GSR Journal

Georgetown Scientific Research Journal

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GSR Journal
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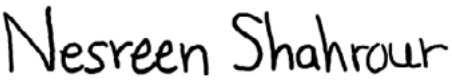
Letter From the Editors

This spring semester marks an unprecedented year for the Georgetown community and beyond. The ability of many student researchers to adapt their research studies to a virtual environment, something previously unimaginable, is nothing short of a remarkable display of the resilience and adaptability of the Georgetown student body. Students turned to writing literature reviews and conducting data analyses to delve deeper into their research virtually. This pattern was reflected in the numerous literature reviews present in this issue as well as in Student Highlights throughout the school year. These unanticipated changes students made to the way they conduct research are inspiring to the research community as a whole and show their passion and desire to further scientific research despite the many new challenges we face.

In this issue, we are met with a diversity of research conducted by these committed students. They have tackled a variety of important topics ranging from gun violence to cancer models. This issue presents many fascinating and thought-provoking studies: a new, original program for decryption techniques on python, a review about the target receptor GPR40 for epilepsy treatment, original research regarding the psychological healing of Black women in the DC area following losing a child to gun violence, and a review of cancer models in pancreatic ductal adenocarcinoma therapy. We hope this issue will serve as a testament to true commitment that will always find a way to move forward despite a pandemic that challenged the safety of conducting research via traditional methods.

As we transition into our second year as an organization, we hope to continue contributing to the scientific student experience at Georgetown University. To honor our dedication to the Georgetown research community, the Center for Student Engagement presented GSR Journal with the Outstanding Student Organization Award. Through resources on how to get involved in research and showcasing the work of our community members, we hope students will learn more about the myriad research opportunities at Georgetown. Please join us in commending the students who have advanced the ongoing research at Georgetown University both in this issue and beyond.

Danya A. Adams
Editor-in-Chief



Nesreen Shahrour
Executive Editor

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An Investigation Into the Mathematics of Decryption Techniques in RSA Encryption, With an Implementation in Python

Sofia Flynn

An Investigation Into the Mathematics of Decryption Techniques in RSA Encryption, With an Implementation in Python

Sofia Flynn

Department of Mathematics, Georgetown University, Washington D.C.
E-mail: smf133@georgetown.edu
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Abstract

This study explores the mathematics of two different techniques that can be used to access the decryption key in RSA encryption including semi-prime factorization and a logarithmic method. The study then presents a Python program, written by the author, that automates the calculations for either of the decryption techniques and also calculates the number of iterations required to determine the decryption key in either circumstance. Most importantly, the program utilizes only values of the RSA encryption algorithm that would be made publicly available in actual circumstances to calculate the decryption key so as to mimic real-life occurrences with as much integrity and accuracy as possible.

Keywords: RSA encryption, semi-prime factorization, decryption, Python

1. Introduction

RSA encryption was created in the 1970s as an asymmetric (public-key) form of cryptography that would replace the then-commonly utilized and weakening symmetric cryptography.[1] In symmetric cryptography, in order to exchange secret messages, two individuals would either have to meet beforehand to exchange identical keys or run the risk of their keys being intercepted.[2] In a simplified example of symmetric cryptography, suppose "Bob" wanted to send a secret message to "Alice." Alice and Bob would first have to meet each other to share identical keys that would be used for encryption and decryption, ensuring that each person ultimately possesses the same key. Using one of the identical keys, Bob could then encrypt his secret message and send it to Alice, who would then decrypt the message using the other identical key that she and Bob had exchanged beforehand. If Bob and Alice cannot meet to share keys, then Bob would have to send the key that decrypts his encrypted message along with his encrypted message to Alice, running the risk that a third party could easily intercept both the encrypted message and key before it comes into Alice's possession.

In 1970, James Ellis proposed a hypothetical idea in which secret messages could be exchanged between individuals without them having to meet beforehand to share identical keys, thereby eliminating dependence on the ever-weakening symmetric cryptography.[3] The premise was simple: if Bob wished to send a secret message to Alice, Alice could create a lock and a key that decrypted, or "unlocked', that lock. Alice could then send the open lock to Bob, who would proceed to encrypt, or "lock", his secret message using the open lock. Alice would retain possession of the key that decrypted the lock. Bob would then send his encrypted message to Alice, who would decrypt it using the key that remained in her possession throughout the entire process. Such is the conceptual idea behind asymmetric

cryptography; by preventing an initial exchange of keys, one avoids the risk of a third party intercepting the key used to decrypt the encrypted message.

In 1973, Clifford Cocks invented a mathematical implementation of Ellis' idea, which had remained classified for twenty-four years.[4] Later on in 1977, Ron Rivest, Adi Shamir, and Leonard Adleman created the equivalent of Cocks' implementation of Ellis' idea completely independently.[5] The algorithm they created based on Ellis' conceptual idea about asymmetric cryptography was henceforth known as RSA (each of the founders' initials) encryption and is widely used today. RSA has formidable strength; as of yet, the only device that is considered to be capable of breaking RSA is a quantum computer.[6]

Rivest, Shamir, and Adleman's algorithm consists of six values that correspond to either a public or private key. The public key is released publicly and can be used by anyone to encrypt a message; the private key, on the other hand, is kept hidden by the "key generator" (the person who creates the public and private key values; the equivalent of "Alice" in the latter example above) and is used by the key generator to decrypt any encrypted message sent to him or her. The six values generated by the key generator are denoted: P, Q, N, φN, E, and D. P, Q, φN, and D belong to the private key and are kept secret by the key generator. N and E belong to the public key and are released publicly.

The key generator must generate values for P, Q, N, φN, E, and D. The key generator must first choose two large prime numbers P and Q. In real world utilizations of RSA encryption, these prime numbers are typically large, usually hundreds of digits long. The key generator must then multiply these two prime numbers together to attain the value of N, that is, P x Q = N. N is a semi-prime number, or the product of two prime numbers. Next, the key generator calculates the φ or "phi" of N. The phi of a number outputs how many positive integers are less than that number that do not share any common factors greater than one with the number. For example, to find the phi of nine, a key generator would list all of the integers less than nine but greater than or equal to one (eight, seven, six, five, four, three, two, one). From this list, they would then evaluate which integers share common factors with nine that are greater than one. Six and three both share common factors greater than one with nine while eight, seven, five, four, two, and one do not share any common factors greater than one with nine. The phi of nine is six because there are six integers less than nine that do not share any common factors greater than one with nine. Calculating the phi of N is straightforward for the key generator due to the fact that the phi function is multiplicative, meaning that:

$$\varphi N = \varphi P \times \varphi Q \text{ (equation 1)}$$

or the phi of N is equivalent to the phi of each of N's factors multiplied together. This property can be proved using the Chinese Remainder Theorem but such a proof is omitted in this paper.[7] Thus, the key generator can calculate the phi of P and the phi of Q and multiply these two values together to attain the phi of N. The phi of any prime number is simply one less than the original prime number because the only factors that prime numbers have are one and itself.[8] For example, the phi of five (a prime number) is four because there are four positive integers less than five that do not share any common factors greater than one with five. Thus, the phi of the prime number P is (P – 1) and the phi of the prime number Q is (Q – 1). Thus, the phi of the semi-prime N is (P – 1)*(Q – 1). To generate E, the key generator must pick an integer that satisfies the requirement: $1 < E < \varphi N$; E shares no common factors with φN. Finally, to generate D, the key generator must pick an integer that satisfies the requirement:

$$(E^*D) \bmod \varphi N = 1 \text{ (equation 2)}$$

The operation "mod" outputs the remainder of a division. For example, 8 mod 6 = 2 because the remainder of the division eight divided by six is two.

Once the key generator has generated values for P, Q, N, φN, E, and D, they must release the values of N and E (the public key values) to the

public but retain the values of P, Q, φN, and D (the private key values). If a person (the "sender"; the equivalent of "Bob" in the latter example above) wishes to send a secret message to the key generator, they can use the values of N and E to encrypt their message using the expression: M^E mod N, where M is the secret message to be encrypted. The sender would then send this encrypted message M^E mod N (the result of which is known as "C", for ciphertext message) to the key generator. The key generator can then decrypt the encrypted message C by using the private key value of D through the expression: C^D mod N. The result of C^D mod N yields the original message M. Thus, in summary:

$$M^E \text{ mod N} = \text{C (equation 3)}$$

and

$$C^D \text{ mod N} = \text{M (equation 4)}$$

with proof of correctness derived by Rivest, Shamir, and Adleman using Fermat's Little Theorem in their publication.

As can be seen, RSA encryption functions similarly to the simplified example of asymmetric encryption given earlier (Introduction, paragraph two). Alice, the key generator, creates a public and private key containing the values of P, Q, N, φN, E, and D. N and E function as the values that Bob uses to lock the open lock that Alice created and sent to him, while the values of P, Q, φN, and D function as the key that opens the lock once Bob locks it with his message and sends it to Alice.

Although publicly releasing the values of N and E does not detract from the strength of asymmetric RSA encryption, it is integral that the values of P, Q, φN, and D remain hidden and can only be accessed by the key generator: this is because RSA encryption was built around two main assumptions that make it safe and acceptable to release the values of N and E publicly. First, it is exceedingly time-consuming to calculate the decryption key (D) by factoring large semi-prime numbers. Second, it is exceedingly time-consuming to calculate the decryption key (D) using properties of logarithms because of the discrete logarithm problem. If the decryption key D was accessed by a person other than the key generator, then this person would be able to intercept secret encrypted messages meant for the key generator and decrypt them using the intercepted value of D. In short, RSA is a secure cryptosystem because accessing the decryption key by semi-prime factorization or logarithmic properties both prove to be too time-consuming to be pursued realistically, at least until the advent of quantum computing.

If a "message-interceptor" (the equivalent of a "hacker") wants to calculate the value of the decryption key using only the publicly-available values of N and E, they have two options, with the first being semi-prime factorization.[9] When semi-prime factorization is used by a message-interceptor in an attempt to gain access to the decryption key, the magnitude of the semi-prime number proves to be of paramount importance in preventing the message-interceptor from calculating the decryption key. In the case of RSA encryption, the semi-prime number is N (the product of the two prime numbers P and Q), whose value is publicly available to the message-interceptor. The message-interceptor must factor the semi-prime number N into its constituent factors P and Q. From there, the message-interceptor can plug in the values of P and Q into the expression (P – 1)*(Q – 1) to find φN. After having attained the value of φN, the message-interceptor, knowing that (E*D) must equal 1 more than φN in order to achieve a modular relationship with 1 as a remainder, can plug this value along with the public key value of E into equation 2 and solve for D. For example, if E is 7 and φN is 3120, then the formula would be: 7D mod 3120 = 1, and thus, 7D must equal 3121 for the relationship to work. With this in mind, the message-interceptor can easily solve for D:

$$7D = 3121$$

$$D = 445.857143 \ldots$$

D, however, must be an integer value. In order to satisfy this rule, the message-interceptor can look for other modular relationships that yield a remainder of 1. For example, 6241 mod 6240 =1.

Similarly, 9361 mod 9360 = 1. The message-interceptor can keep on multiplying the value of 3120 by increasing integers to achieve different modular relationships that will still yield 1 as a remainder until D is an integer value.

Example:

7D mod (3120*1) = 1; 7D = 3120*1 + 1; D = (3120*1 + 1)/7; D = decimal

7D mod (3120*2) = 1; 7D = 3120*2 + 1; D = (3120*2 + 1)/7; D = decimal

7D mod (3120*3) = 1; 7D = 3120*3 + 1; D = (3120*3 + 1)/7; D = decimal

7D mod (3120*4) = 1; 7D = 3120*4 + 1; D = (3120*4 + 1)/7; D = integer

Here, solving for D only requires 4 iterations. The formula for solving for D simplifies to:

D = (φN* φNMultiplier + 1)/E (equation 5)

where "φNMultiplier" is a specific integer value that the message-interceptor multiplies φN by to achieve different modular relationships that yield 1. With knowledge of the publicly-available value of E as well as the value of φN, the message-interceptor can easily calculate the value of the decryption key, D, having created one equation with only one unknown value. However, one problem exists: factoring N into its constituent factors P and Q becomes increasingly time-consuming as N grows. This is why large prime numbers had to be chosen by the key generator as values for P and Q. The larger N is (the product of the already large values of P and Q), the more time it will take to factor it. In RSA, the types of semi-prime numbers utilized are typically around 600 digits long. Semi-prime numbers of such magnitudes take a huge amount of time to factor using current technologies. In fact, in 1978, Rivest, Shamir, and Adleman predicted that factoring a 500-digit semi-prime number would take approximately 4.2 x 10^{25} years to factor using the Schroeppel factoring algorithm.[10] Therefore, due to the immense amount of time needed to factor N into its constituent factors P and Q, it is impractical for a message-interceptor to use semi-prime factorization to calculate the decryption key, D.[11]

The second option that the message-interceptor has if he wishes to calculate the value of the decryption key using only the publicly available values of N and E is to use the properties of logarithms. Recall that encrypting a message requires plugging a secret message M into the expression M^E mod N, and thus decrypting that message requires plugging in the result of M^E mod N (which is C) into the expression C^D mod N to attain the original message M. The message-interceptor, like all others, has access to the public key values of N and E. He can generate a secret integer message M and plug it into the expression M^E mod N to attain the ciphertext/encrypted message C. Then, they can plug the value of C along with the public key value of N into the expression C^D mod N. The message-interceptor knows that the result of C^D mod N must equal his original message M (see equation 4). The message-interceptor has knowledge of the value of N (a public value) as well as C and M, because he generated these values. Therefore, the message-interceptor has created an equation in which only one unknown, D, the decryption key, exists. For example, if N is 143 and E is 7, and the message-interceptor picks his secret message M to be 24, then the enciphered message would be 24^7 mod 143, or 106. The message-interceptor can plug in the values of 143 (N), 24 (M) and 106 (C) into equation 4, yielding 106^D mod 143 = 24, and subsequently solve for D. Knowing that 106^D must equal to 143 + 24, or 167, for the above equation to work due to modular arithmetic (167 mod 143 = 24), the message-interceptor can set 106^D to 167. Knowing that 106^D = 167, the message-interceptor can easily solve for D using the properties of logarithms:

$$106^D = 167$$

$$\log 106^D = \log 167$$

$$D (\log 106) = \log 167$$

$$D = \log 167/\log 106$$

$$D = 1.09747 \ldots$$

However, D has the restriction that it must be an integer value, which the above value is not. Thus, the message-interceptor needs to come up with another way to calculate D using this method. The message-interceptor knows that 167 mod 143 = 24, but there are other values that, when divided by each other, will yield a remainder of 24. 143 multiplied by 2 is 286. 286 plus 24 is 310. Thus, 310 mod 286 = 24. Similarly, 143 multiplied by 3 is 429. 429 plus 24 is 453. Thus, 453 mod 429 = 24. The message-interceptor can keep on multiplying the value of 143 by increasing integers to achieve different modular relationships that will still yield 24 as a remainder until D is an integer value.

Example:

106^D mod (143*1) = 24; 106^D = (143*1) + 24; log(106^D) = log(143*1 +24); D = log(143*1 + 24)/log(106); D = decimal

106^D mod (143*2) = 24; 106^D = (143*2) + 24; log(106^D) = log(143*2 +24); D = log(143*2 + 24)/log(106); D = decimal

106^D mod (143*3) = 24; 106^D = (143*3) + 24; log(106^D) = log(143*3 +24); D = log(143*3 + 24)/log(106); D = decimal

The formula for solving for D in this way can be simplified to:

D = log(N*NMultiplier + M)/log(C) (equation 6)

where "NMultiplier" is the increasing integer by which the message-interceptor multiplies N to achieve different modular relationships that will still yield the secret message, M. Even though the message-interceptor can keep on running this type of sequence to achieve an integer value for D, the amount of iterations it would take to finally attain an integer value for D is astronomical, making the logarithmic decryption key calculations just as impractical and time-consuming as semi-prime factorization. The difficulty of calculating the exponent D in this way is known as the discrete logarithm problem, or the "RSA problem."

Decryption through semi-prime factorization and decryption through logarithmic properties are both processes that are referred to as "trapdoor one-way functions".[12] Trapdoor one-way functions are easy to compute in one direction but difficult to compute in the other direction unless special "trapdoor" information is known. In the case of decryption through semi-prime factorization, Rivest, Shamir, and Aldeman realized that it was trivially easy to multiply two large prime numbers P and Q together yet infinitely more difficult and time-consuming to factor the resulting semi-prime number, N. Similarly, they realized that it was easy to calculate M in equation 4 given the values of C, N, and most importantly, D, but far more arduous to calculate the value of D in equation 4 given the values of C, N, and M. These two utilizations of trapdoor one-way functions in RSA encryption are what make it so formidably strong and currently unbreakable without a quantum computer.[13]

In the case of decryption through either semi-prime factorization or logarithmic properties, an immense number of iterations are required to finally attain the decryption key, D. In the case of semi-prime factorization, if every integer less than the semi-prime number, N, and greater than or equal to two was tested to see if it divided evenly into N, the number of iterations required would be N – 2. If N is a huge semi-prime number that is hundreds of digits long, the number of iterations required to factor N would be two subtracted from the huge semi-prime number. While it is true that not all the numbers less than N would have to be assessed if it was certain that N was semi-prime (because this would narrow the numbers that needed to be tested down to only prime numbers), if access to a large bank of prime numbers is not unattainable (as in this project), all integers greater than or equal to two and less than N would still have to be tested to see if they divide evenly into N, making the expression for the amount of iterations needed to factor N in this project N – 2. In the algorithm presented for factoring N in this manner, time complexity is O(N). In the case of decryption through logarithmic properties, $\frac{10^{(logC)*D} - M}{N}$ iterations are required to acquire D. Because the formula for solving for D through

logarithmic techniques can be simplified to equation 6, where "NMultiplier" is the increasing integers the message-interceptor multiplies N by to achieve different modular relationships that will still yield the secret message, M, "NMultiplier" can be isolated and solved for to attain the number of iterations it would take to solve for D. The following demonstrates the process of isolating "NMultiplier" from the equation D = log(N*NMultiplier + M)/log(C):

$$D = \log(N*NMultiplier + M)/\log(C)$$

$$D*[\log(C)] = \log(N*NMultiplier + M)$$

$$10^{\{D*[\log(C)]\}} = N*NMultiplier + M$$

$$10^{\{D*[\log(C)]\}} - M = N*NMultiplier$$

$$NMultiplier = \frac{10^{\{D*[\log(C)]\}} - M}{N} \text{ (equation 7)}$$

By solving for "NMultiplier", the number of iterations needed to calculate D through logarithmic techniques has been calculated to be $\frac{10^{\{D*[\log(C)]\}} - M}{N}$. Because this program allows a maximum of one million iterations to compute D in this manner, the associated time complexity is less than O(1000000).

The purpose of this research study is to discuss the function of a program built using Python that calculates the decryption key using semi-prime factorization as well as the logarithmic method. Further, the program calculates the number of iterations required to calculate D using either of these methods, indicating the efficacy and advantageousness of using one method over the other in different circumstances. This research study will delve into explaining the code written in the program and how it contributes to achieving the end result of decryption as well as the number of iterations derived necessary to calculate D using either of the aforementioned methods, being that of semi-prime factorization or logarithms.

2. Materials

2.1 Required Materials:

Python programming system installed on MacBook laptop or other personal computer, complete with IDLE and running module.
Access to Python's "math" module located in Python's standard library. The module includes mathematical functions necessary to the operation of the program.

2.1 Recommended Materials:

Access to a computational knowledge/answer engine like Wolfram Alpha used to initially verify the accuracy of the results obtained from the program.[14]

2. Methods/Procedures

```
1 Program start
2 Print "DECRYPTION THROUGH SEMI-PRIME FACTORIZATION"
3 Initialize variable N = user-given integer input
4 Initialize list NFactorsList = 1, N
5 Start loop
6               For integers i in range (2, N)
7                       If N mod i = 0
8                               Add i to NFactorsList
9 End loop
10 Start loop
11              While length of NFactorsList ≠ 4
12                      N = user-given integer input
13                      Delete NFactorsList
14                      Add 1, N to NFactorsList
15                      Start loop
16                              For integers i in range (2, N)
17                                      If N mod i = 0
18                                              Add i to NFactorsList
19                      End loop
20 End loop
21 Start loop
22              For integers i in range (2, N)
23                      If N mod i = 0
24                              Initialize variable Q = i
25                              Initialize variable P = N/Q
26                              Initialize variable PhiN = (P – 1)*(Q – 1)
27 End loop
28 Initialize string Pequals = "P = "
29 Initialize string Qequals = "Q = "
30 Initialize string PhiNequals = "Phi N = "
31 Print Pequals followed by P
32 Print Qequals followed by Q
33 Print PhiNequals followed by PhiN
34 Initialize list PhiNFactorsList = 1, PhiN
35 Delete elements 0 – 2 in PhiNFactorsList
36 Start loop
37              For integers i in range (2, PhiN)
38                      If PhiN mod i = 0
39                              Add i to PhiNFactorsList
40 End loop
41 Initialize list PrimesLessThan100List = 2, 3, 5, 7, 11, 13, 17, 19, 23, 29, 31, 37, 41, 43, 47, 53, 59, 61, 71, 73, 79, 83, 89, 97
42 Initialize variable E = 1
43 Start loop
44              While E = 1
45                      If first element of PrimesLessThan100List is not in PhiNFactorsList
46                              E = first element of PrimesLessThan100List
47                      Else
48                              Delete first element of PrimesLessThan100List
49 End loop
50 Initialize string Eequals = "E = "
51 Print Eequals followed by E
52 Initialize variable PhiNMultiplier = 1
53 Start loop
54              While (((PhiNMultiplier*PhiN) + 1)/E) mod 1 does not equal 0
55                      PhiNMultiplier = PhiNMultiplier + 1
56 End loop
57 Initialize variable D = ((PhiNMultiplier*PhiN) + 1)/E
58 Initialize string Dequals = "D = "
59 Print Dequals followed by D
60 Print "DECRYPTION THROUGH LOGARITHMS"
61 Initialize variable M = user-given integer input
62 Initialize variable C = M raised to the E power all mod N
63 Initialize string Mequals = "Secret message = "
64 Initialize string Cequals = "Encrypted message = "
65 Print Mequals followed by M
66 Print Cequals followed by C
67 Initialize list NonIntegerDList = 1, 2
68 Delete all elements of NonIntegerDList
69 Initialize variable NMultiplier = 1
70 Initialize variable logOfC = log(C)
71 Start loop
72              While NMultiplier does not equal 1000000
73                      If ((log(N*NMultiplier + M))/(logOfC)) mod 1 does not equal 0
74                              Add ((log(N*NMultiplier + M))/(logOfC)) to NonIntegerDList
75                              NMultiplier = NMultiplier + 1
76                      Else
77                              D2 = ((log(N*NMultiplier + M))/(logOfC))
78 End loop
79 If D2 = 1
80              Print "D = More than a million iterations to calculate"
81 Else
82              Print D2
83 Print "HOW MANY ITERATIONS?"
84 Initialize string ThroughSemiPrimeFactorization = "Iterations using semi-prime factorization = "
85 Initialize string ThroughLogarithms = "Iterations using logarithms = "
86 Initialize variable IterationsViaSemiPrimeFactorization = N – 2
87 Print ThroughSemiPrimeFactorization followed by ItrcationsViaSemiPrimcFactorization
88 Initialize variable IterationsViaLogs = ((10 to the (logOfC*D)) – M)/N
89 Print ThroughLogarithms followed by IterationsViaLogs
```

Figure 1. Code script

3.1 The Code Behind Semi-Prime Factorization to Calculate D (Lines 1-47)

3.1.1 Factoring N

In lines 3 – 9, the program asks for a semi-prime input which it then sets to the variable N. The program is designed to test all integers less than N but greater than or equal to two to determine whether they divide evenly into N. An integer is a factor of N if the remainder of N divided by said integer equals zero. The integers that do divide evenly into N are deemed to be factors of N and are appended to a list called "NFactorsList".

In lines 10 – 20, the program forces another input to be given for N if the first input is not a semi-prime number. If the length of NFactorsList is only four, this indicates that N is a semi-prime number because semi-prime numbers only have four factors: one, the semi-prime itself, and the two prime numbers multiplied together to attain the semi-prime number.

In lines 21 – 25, after ensuring a semi-prime input for N, the program sets the variables P and Q to the two prime numbers that N is divisible by.

3.1.2 Factoring φN

In lines 26 – 27, the program sets the variable PhiN to (P – 1)*(Q – 1) based on the mathematics described in the introduction for the calculation of φN.

3.1.3 Generating a public key value for E

In lines 34 – 40, the program initializes a list called "PhiNFactorsList" that lists the factors of the value of φN by testing which integers are greater than two but less than φN and yield a remainder of zero when divided into φN.

In lines 41 – 42, the program initializes a list called "PrimesLessThan100List" which lists all of the prime numbers less than 100 and also initializes that variable E by setting it to a placeholder value of 1.

In lines 43 – 49, the program generates a value for E, which must be greater than 1, less than φN, and share no common factors with φN. The program cross-checks values in the PrimesLessThan100List against values in the PhiNFactorsList until it finds a value in the PrimesLessThan100List that is not in the PhiNFactorsList, which it then sets it to the variable E. By checking primes as possible values for E against the factors of φN, the program ensures that whatever value it picks for E shares no common factors with φN.

3.1.4 Generating D

In lines 52 – 56, the program sets a variable called "PhiNMultiplier" to 1, which will act as φNMultiplier in equation 5. While D does not equal an integer solution, the program continuously adds 1 to the value of PhiNMultiplier. When PhiNMultiplier iterates enough that it yields an integer value for D, the program sets D to (φN* φNMultiplier + 1)/E, where φNMultiplier represents the number of iterations it took to yield an integer solution for D.

3.2 The Code Behind Logarithmic Techniques to Calculate D (Lines 48 – 71)

In lines 61 – 62, the program asks for an integer input and sets it to the variable M. It then encrypts M by raising it to the value of E, dividing the entire expression by N, and taking the modulus of the division, which is C, the ciphertext message. In lines 67 – 82, the program initializes a variable called "NMultiplier" to 1 which acts as NMultiplier in equation 6. Because Python has a limited capacity for running large numbers of iterations, the program continuously adds 1 to NMultiplier until NMultiplier = 1000000 (the first million iterations) instead of until D is an integer. When NMultiplier = 1000000, although D might not be an integer, the program still cuts off at this point and prints that D takes more than one million iterations to calculate. All non-integer numbers resulting from log(N*NMultiplier + M)/log(C) are appended to the list "NonIntegerDList" and stored.

3.3 The Code Behind Iterations for Semi-Prime Factorization to Calculate D and Logarithmic Techniques to Calculate D (Lines 72 – 80)

In lines 86 – 89, the iterations for decryption using semi-prime factorization and for decryption using logarithmic techniques are both calculated using the expressions N – 2 and $\frac{10^{(logC)*D} - M}{N}$, respectively (see introduction of derivation of iterations). The program then prints the number of iterations needed in either case, allowing for quick comparison of either method's effectiveness in different circumstances.

4. Results

```
1  DECRYPTION THROUGH SEMI-PRIME FACTORIZATION

2  Enter a semi-prime number: 1500
3  Your prior input was not a semi-prime number. Enter a semi-prime number: 1820
4  Your prior input was not a semi-prime number. Enter a semi-prime number: 143
5  P = 11
6  Q = 13
7  Phi N = 120
8  E = 7
9  D = 103

10 DECRYPTION THROUGH LOGARITHMS

11 Enter a secret message to encrypt: 24
12 Secret message = 24
13 Encrypted message = 106
14 D = More than a million iterations to calculate

15 HOW MANY ITERATIONS?

16 Iterations using semi-prime factorization = 141
17 Iterations using logarithms = 2.82597349859519e+206
```

Figure 2. Program yield

The creation of the Python code described on the previous pages (Figure 1) resulted in a program that, when run, yielded the above result (Figure 2). Lines 1 – 9 print each of the values necessary to calculate the decryption key, D, through semi-prime factorization. In line 2, the program asks for a semi-prime number input. If a number that is not semi-prime is inputted, as in Figure 2 when 1500 was inputted, the program asks for another semi-prime value instead, displaying the message "Your prior input was not a semi-prime number.

Enter a semi-prime number:" until a semi-prime value has been given. When a semi-prime value is given, this number is factored into its constituent factors, P and Q, which are then printed. φN is then calculated by plugging P and Q into the expression (P – 1)*(Q – 1) and subsequently printed. Once a public key value E is generated and printed, D is solved for in equation 2 and printed. Lines 10 – 14 print the necessary information to calculate D through logarithms. In line 11, the program asks for an integer message M, prints M in line 12, prints C (the value of M, encrypted) in line 13, and prints D in line 14 by solving for it in equation 4. However, in all the times that the program has been run, it has never been able to calculate D through logarithms due to the fact that it would take an incredible amount of iterations to do this. Instead, the program prints, "D = more than a million iterations to calculate" if it cannot calculate D through logarithms in under a million iterations. Finally, in lines 15 – 17, the program prints the number of iterations that the program needs to calculate D in either instance. On occasion, the program will experience an overflow error when it tries to calculate the iterations for decryption through logarithms because Python does not have the capacity for such huge calculations.

This project would be economically feasible to implement as it would not cost anything to download an app version of this program onto a mobile device once a graphical user interface has been created for all of the code that has been written.

5. Discussion and Conclusions

The research study undertaken was largely successful because it met all of the objectives defined in the introduction: to describe the mathematics of decryption techniques, to create a program capable of calculating the decryption key in RSA using two different methods and calculating the number of iterations required to accomplish this in either instance, and to explain the code written in the program and its roles in ultimately achieving the end result of decryption. The program has the capacity to calculate the decryption key through both semi-prime factorization and through the logarithmic method. That being said, the logarithmic method almost always takes too many iterations to feasibly calculate the decryption key because of the exponentiation operations involved. However, the program is still theoretically capable of calculating the decryption key through the logarithmic method. The program is also capable of calculating the number of iterations needed to calculate D through either method but will at times experience an overflow error if the number of iterations needed to calculate D through logarithmic techniques exceeds Python's programming capacity. If the code in this study can be replicated using more powerful and faster programs, it would be surmised that D would be calculated with more rapidity through the logarithmic technique and the program would not experience overflow errors. When such errors occur, exception handling can be used to catch the errors using Python's OverflowError. Such an implementation is provided in the appendix.

Additionally, there are many other techniques in existence to factor large semi-prime numbers, though this program only makes use of one of the most rudimentary such techniques. The quadratic sieve and the general number field sieve are two such integer factorization algorithms that far outstrip the technique presented in this research.[15]

Further, this program could have possible implementations in the future. Because it is capable of calculating the number of iterations needed to obtain the decryption key through either semi-prime factorization or logarithms, the program could potentially be used as a starting point to create an entirely new study in which the efficacy of either decryption method is compared when certain variables like the length of the semi-prime number N or the public exponent E are varied.

Acknowledgements

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Appendix

An implementation of the program in pseudocode is provided below in which exception handling is used to handle overflow errors.

```
Program start
Print "DECRYPTION THROUGH SEMI-PRIME FACTORIZATION"
Initialize variable N = user-given integer input
Initialize list NFactorsList = 1, N
Start loop
        For integers i in range (2, N)
                If N mod i = 0
                        Add i to NFactorsList
End loop
Start loop
        While length of NFactorsList ≠ 4
                N = user-given integer input
                Delete NFactorsList
                Add 1, N to NFactorsList
                Start loop
                        For integers i in range (2, N)
                                If N mod i = 0
                                        Add i to NFactorsList
                End loop
End loop
Start loop
        For integers i in range (2, N)
                If N mod i = 0
                        Initialize variable Q = i
                        Initialize variable P = N/Q
                        Initialize variable PhiN = (P – 1)*(Q – 1)
End loop
Initialize string Pequals = "P = "
Initialize string Qequals = "Q = "
Initialize string PhiNequals = "Phi N = "
Print Pequals followed by P
Print Qequals followed by Q
Print PhiNequals followed by PhiN
Initialize list PhiNFactorsList = 1, PhiN
Delete elements 0 – 2 in PhiNFactorsList
Start loop
        For integers i in range (2, PhiN)
                If PhiN mod i = 0
                        Add i to PhiNFactorsList
End loop
Initialize list PrimesLessThan100List = 2, 3, 5, 7, 11, 13, 17, 19, 23, 29, 31, 37, 41, 43, 47, 53, 59, 61, 71, 73, 79, 83, 89, 97
Initialize variable E = 1
Start loop
        While E = 1
                If first element of PrimesLessThan100List is not in PhiNFactorsList
                        E = first element of PrimesLessThan100List
                Else
                        Delete first element of PrimesLessThan100List
End loop
Initialize string Eequals = "E = "
Print Eequals followed by E
Initialize variable PhiNMultiplier = 1
Start loop
        While (((PhiNMultiplier*PhiN) + 1)/E) mod 1 does not equal 0
                PhiNMultiplier = PhiNMultiplier + 1
End loop
Initialize variable D = ((PhiNMultiplier*PhiN) + 1)/E
Initialize string Dequals = "D = "
Print Dequals followed by D
Print "DECRYPTION THROUGH LOGARITHMS"
Initialize variable M = user-given integer input
Initialize variable C = M raised to the E power all mod N
Initialize string Mequals = "Secret message = "
Initialize string Cequals = "Encrypted message = "
Print Mequals followed by M
Print Cequals followed by C
Initialize list NonIntegerDList = 1, 2
Delete all elements of NonIntegerDList
Initialize variable NMultiplier = 1
Initialize variable logOfC = log(C)
Start loop
        While NMultiplier does not equal 1000000
                If ((log(N*NMultiplier + M))/(logOfC)) mod 1 does not equal 0
                        Add ((log(N*NMultiplier + M))/(logOfC)) to NonIntegerDList
                        NMultiplier = NMultiplier + 1
                Else
                        D2 = ((log(N*NMultiplier + M))/(logOfC))
End loop
If D2 = 1
        Print "D = More than a million iterations to calculate"
Else
        Print D2
Print "HOW MANY ITERATIONS?"
Initialize string ThroughSemiPrimeFactorization = "Iterations using semi-prime factorization = "
Initialize string ThroughLogarithms = "Iterations using logarithms = "
Initialize variable IterationsViaSemiPrimeFactorization = N – 2
Print ThroughSemiPrimeFactorization followed by IterationsViaSemiPrimeFactorization
Try
        Initialize variable IterationsViaLogs = ((10 to the (logOfC*D)) – M)/N
        Print ThroughLogarithms followed by IterationsViaLogs
Except
        Print "Overflow error.  Computing iterations using logarithms exceeds programming capacity."
```

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GPR40 and Postsynaptic NMDA Receptors: A Pair Against Epilepsy

Vidya Mullangi,* Nikita Shah,* Hyunwook Nam,* Alexa Asch,*
Lauren Cox, Clarisa Mendoza
*Indicates equal contribution

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Vidya Mullangi*, Nikita Shah*, Hyunwook Nam*, Alexa Asch*, Lauren Cox, Clarisa Mendoza

*Indicates equal contribution
Department of Biology, Georgetown University, Washington D.C.
E-mail: vm491@georgetown.edu, ns1217@georgetown.edu, hn210@georgetown.edu, aa2142@georgetown.edu, lec122@georgetown.edu, cem328@georgetown.edu
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Abstract

Epilepsy is a chronic neurological condition characterized by abnormal brain activity, unusual behavior, and loss of awareness. One of the most common features is the spontaneous recurrence of unprovoked seizures that mainly affect the hippocampus and cortical regions of the brain. Although the exact cause of epilepsy is still unknown, a mix of genetic, neurological, and environmental factors play a role. A novel study by Yang et al. explores the metabotropic receptor GPR40 which is suspected to be involved in the regulation of epileptic seizures, specifically through its modulatory role on NMDA receptors in the central nervous system. Their findings suggest that GPR40 induces NMDA receptor endocytosis via direct interaction with NR2A and NR2B subunits of postsynaptic NMDA receptors. Through this mechanism, NMDA-mediated postsynaptic currents are altered, resulting in reduced seizure-like activity. This review article discusses these novel findings which not only shed light on the potential molecular mechanisms of epilepsy but also push the scientific community closer to developing a treatment for this disorder.

Keywords: Epilepsy, GPR40, seizures, NMDA receptors, NR2A and NR2B subunits, excitatory postsynaptic currents, cortex, and hippocampus

1. Introduction

Epilepsy is a chronic neurological condition that affects an estimated one to three percent of the population; it is characterized by the spontaneous recurrence of unprovoked seizures, seizures that occur without apparent triggers.[1] Epilepsy can also be characterized by abnormal brain activity, unusual behavior or sensations, and even a loss of awareness, though these are often common characteristics of other neurological disorders as well.[2] Other general symptoms of epilepsy may include temporary confusion, uncontrollable motor function, loss of consciousness, and even psychological symptoms, such as anxiety, fear, or deja vu.[2]

The exact cause of epilepsy has not yet been determined. However, the neurological disorder has been connected to a variety of factors.[2] The onset of epilepsy has been associated with several developmental disorders including autism and neurofibromatosis. It has also been associated with infectious diseases such as meningitis, acquired immunodeficiency syndrome, and viral encephalitis. Brain trauma caused by stroke or brain tumors can also lead to the onset of epilepsy; in fact, stroke is known to be a common cause of epilepsy in individuals over thirty-five years old.[2]

Additionally, head trauma and traumatic brain injury can play a role in the development of epilepsy. Lastly, research has supported links between epilepsy and specific genes in the human genome. Some genes may contribute to making an individual more susceptible to environmental conditions that might trigger seizures, making genetic influence another factor in the development of epilepsy.[2]

The relationship between genetic influence, gene expression, and protein synthesis has shed light on a new protein, G-protein coupled receptor 40 (GPR40), that is involved in the regulation of epileptic seizures, specifically through its role in regulating NMDA receptors in the central nervous system.[3] The novel study conducted by Yang et al, "GPR40 modulates epileptic seizure and NMDA receptor function," investigated the impact of GPR40 on epileptic brains, and more specifically, how expression of GPR40 affected NMDA receptor function and NMDA receptor-mediated synaptic transmission. This study determined the specific localization of GPR40 in the brain and central nervous system, how the upregulation of the receptor affects epileptic seizures, and how NMDA receptor mediated synaptic transmission function is affected, through regulation of specific subunits of the NMDA receptor on the postsynaptic neuron.[3]

Various antiepileptic drugs (AEDs) have been synthesized to combat seizure activity in epileptic individuals. A meta-analysis reviewing the efficacy of 11 antiepileptic drugs administered to over 900 patients revealed that pregabalin, tiagabine, and vigabatrin yielded the most significant reductions in seizures (>50%).[5] However, similar meta-studies have revealed alternative drugs as the most effective, taking factors such as adverse side effects and tolerability of the drugs into account. While many of these AEDs have adequate efficacy, 30% of patients using these drugs continue to experience some side effects of the treatment, including insomnia, depression, and dizziness.[3] These antiepileptic drugs target neurotransmission, specifically to maintain the balance of excitatory and inhibitory neurotransmission that is normally disrupted in epileptic brains.[3] For the development of a more effective antiepileptic drug that can both treat and limit the side effects of epileptic seizures, many factors must be taken into account, including the neurological root of the disorder. The investigation conducted by Yang et al. aims to obtain a deeper understanding of the root cause of epileptic seizures and the onset of epilepsy.[3] These studies may contribute to the future development of a more effective cure for this chronic neurological condition.

As aforementioned, epilepsy is characterized by excessive and abnormal neuronal activity. The precise mechanism is yet to be discovered as epilepsy is known to have multiple pathophysiological causes including genetic mutation, traumatic brain injury, and exposure to toxins. However, the imbalance of excitatory and inhibitory neurotransmitters is understood as the most common mechanism of various epileptic seizures.[6] To elaborate, seizures are often the result of sudden synchronized neuronal signaling and changes in inhibitory and excitatory stimulation. Many neurotransmitters and hormones such as serotonin, norepinephrine, histamine, are involved in epilepsy.[7] Gamma-aminobutyric acid (GABA) and nicotinic acetylcholine (ACh) receptors, in particular, have been implicated. Mutations in GABA receptors have been shown to cause ER stress and ion imbalance, contributing to epileptogenesis. Additionally, dysfunctional acetylcholine signaling has been shown to aggravate inflammation, a key feature of epilepsy.[7]

Although epilepsy can occur in any region of the brain, it is known to affect the frontal lobe and the temporal lobe most commonly, particularly the hippocampus.[8] Therefore, studying the regulatory mechanisms of neurotransmitters in cortical and hippocampal regions of the brain is important in furthering our understanding of epilepsy. Interestingly, recent studies have found that GPR40 plays a role in modulating synaptic transmission, especially in the cortex and hippocampus.[3] Therefore, the Yang et al. study

was set out to investigate the role of GPR40 in epileptic seizure.[3]

2. Description of GPR40 Involvement in Regions of the Brain

While previous studies have shown that GPR40 is expressed in the cortex and hippocampus, the precise distribution and expression levels are poorly understood.[9] The novel study by Yang et al., demonstrated the distribution and expression levels of GPR40 in both normal and epileptic rodent brains through immunofluorescence staining.[3] This methodology revealed that GPR40 was highly expressed in the lacunosum moleculare layer and the pyramidal cell layer of the hippocampus, while it was not highly expressed in the dentate gyrus. The study further evaluated the cellular level localization of GPR40 from a kainic acid (KA) induced temporal lobe epilepsy (TLE) model and normal control brain. GPR40 was colocalized with microtubule-associated protein 2 (MAP2; a marker of dendrites) and postsynaptic density-95 (PSD95; a postsynaptic marker) but not with glial fibrillary acidic protein (GFAP; an astrocyte marker) in the hippocampus of both the epileptic brain and normal control brain.[3] These results suggested that GPR40 is mostly expressed in postsynaptic excitatory neurons but not in astrocytes.[3]

Expression levels of GPR40 in the hippocampus of epileptic and nonepileptic tissues were compared to validate that GRP40 expression is meaningfully correlated with epilepsy. Immunofluorescence signals of GPR40 were significantly increased in the CA1 hippocampal region of the epileptic rodent brain model compared to those of the nonepileptic control mice. Consistent with this mouse model, high GPR40 expression was observed in the human neocortex of TLE patients. In the study, the team conducted a western blot experiment to further validate that GPR40 protein expression increased in epileptic rodent brain models compared to normal control brains. Congruent with the previous results, the epileptic brain models showed significantly higher GPR40 expression in the cortex and hippocampus compared to the control.[3] Elevated GPR40 levels in epileptic brains suggest a possible role of GPR40 in modulating epilepsy.

Therefore, the experiments suggest that GPR40 may be involved in epilepsy. However, further experiments must be conducted in order to discover the direct relationship between GPR40 and epilepsy. If GPR40 directly downregulates or upregulates epileptic seizures, then it is crucial to further study its regulatory mechanisms and signaling pathway.

3. NMDAR-Mediated GPR40 Signaling Modulates Epileptic Seizures

Also known as the Free Fatty Acid Receptor 1, GPR40 is bound and activated by long, unsaturated fatty acids called PUFAs. The existing literature has argued that PUFAs may have differential effects in the CNS.[10, 11] In fact, one group in particular, omega-3 PUFAs, has been shown to have anticonvulsant effects. A study by the University of Toronto's Epilepsy Research Program found that various fatty acid chains, including ALA, EPA, and DHA, reduced the frequency of action potentials and excitatory signaling *in vitro*.[10] However, they posited that the observed effects were modulated through voltage-gated Na^+ and Ca^{2+} channels, further supporting that seizure-like activity is dependent on a plethora of signaling pathways and factors. In contrast, other studies present confounding results, showing that low doses of certain PUFAs reduce seizure frequency whereas increased doses have no significant effects on seizure frequency.[11] PUFAs may therefore act on many target locations, either reducing or increasing seizure-like activity. These conflicting results reveal the need for further research to fully understand the various, complex factors that modulate epileptic activity.

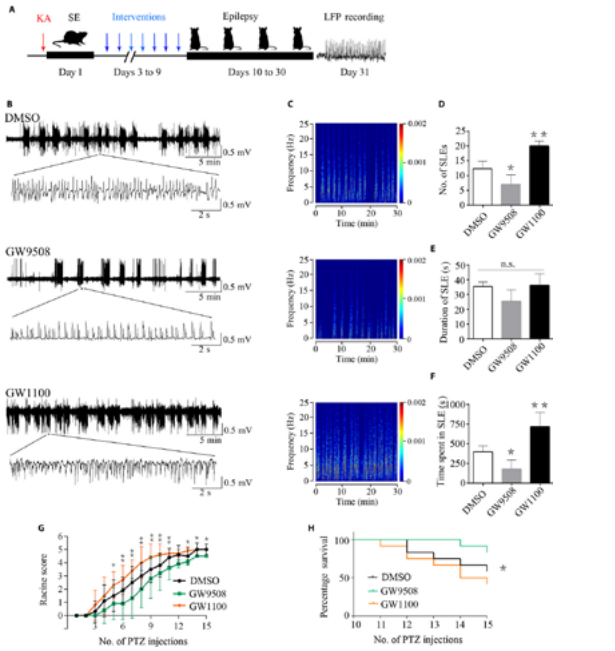

Figure 1: Activation of GPR40 via KA-induced and PTZ kindling models Yields Highest Survival Rates. a) Representation of KA-induced experimental timeline. After KA injection, mice were treated with DMSO, GW9508, or GW1100 (n=6 in each group). b) LFP recordings from each treatment group over 5 minutes and zoomed in of 2 s. c) Corresponding frequency recordings of LFP. d-f) Comparing three groups in number, duration, and time spent in SLEs. g) Using the PTZ model, seizure activity was heightened in GW1100 and reduced in GW9508 groups. h) Highest survival amongst GW9508 group and lowest survival amongst GW1100 group (Figure taken from Yang et al., 2018).

Yang et al. discovered that GPR40 activation results in a layered cascade of signaling events. Overall, GPR40 affected NMDA receptor endocytosis by binding to NR2A and NR2B subunits found on neuronal membranes. To elaborate, the research team needed to confirm that GPR40 played a direct, causal role in modulating epileptic seizures. In order to accomplish this initial goal, they utilized an intrahippocampal KA-induced TLE model, meaning that KA was injected into mice's hippocampi unilaterally to induce seizures (Figure 1A). Previous studies have shown that KA, an excitatory amino acid, is a useful tool for seizure-induction because it induces certain seizures that are commonly experienced by patients with temporal lobe epilepsy.[12] Three days after the induction of epileptic activity, additional compounds were injected daily for one week. These treatments included a DMSO control, GW9508 agonist, or GW1100 antagonist of the target GPR40 receptor. After one-month, local field potentials, which were characterized as strong electrical signals between neurons, were measured (Figure 1B). These measurements reflected both the frequency and duration of any seizure-like events (SLE's) experienced by the mice. The compiled data revealed that mice injected with the GW1100 antagonist experienced more seizure-like events that lasted longer compared to the control (Figure 1D, F). In contrast, mice injected with the GW9508 agonist experienced fewer seizure-like events that lasted a significantly shorter time compared to the control (Figure 1D, F).[3]

To confirm their findings, the researchers repeated their experiment with a pentylenetetrazol (PTZ) kindling model. Like kainic acid, pentylenetetrazol, a GABA A receptor antagonist, was used to induce convulsive activity in the mice[13]; this model therefore represents the seizure-like symptoms experienced by many patients suffering with epilepsy. Like the previous model, the mice received intracerebroventricular injections of one of three treatments: the DMSO control, the GW9508 agonist, or the GW1100 antagonist. While all three mice groups showed increased seizure scores after PTZ treatment, the mice treated with GPR40 selective agonist exhibited significantly lower seizure scores compared to those treated with GPR40 antagonist. As PTZ injections continued to be administered, the mice treated with the agonist had the highest survival rate, while the mice treated with the antagonist had the lowest survival rate and were most prone to generalized tonic-clonic seizure (GTCS) related death (Figure 1G, H). These findings served as a key indication that activating or inhibiting GPR40 receptors directly affects epileptic seizure activity; specifically, activating the receptor resulted in reduced epileptic activity and an increased chance

for survival, while inhibiting the receptor led to opposite observed effects.[3]

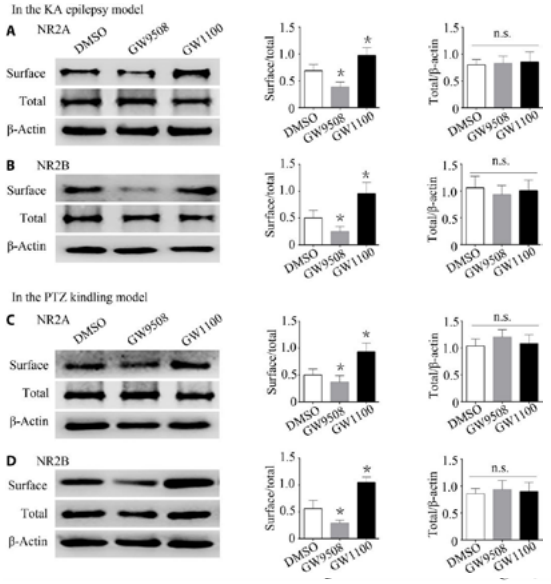

Figure 2: Changes in Cell Surface NR2A and NR2B Subunit Expression in Hippocampal Tissue Samples in GPR40-treated Mice during Epilepsy. After KA injection or PTZ treatment, mice were treated with DMSO, GW9508, or GW1100 (n=5 in each group). a-b) KA model: cell surface and total N2RA and NR2B expression was quantified via western blot. c-d) PTZ model: cell surface and total NR2A and NR2B expression was quantified via western blot. Significant differences were observed in both treatment models between antagonist and agonist groups. One-way ANOVA and Tukey's Test (Figure taken from Yang et al., 2018).

The next aim was to determine how GPR40 regulated seizure-like activity. They found that GPR40 regulated the functions of NR2A and NR2B as well as NMDAR-mediated synaptic responses. Interestingly, the different subunits of the NMDA receptor are referred to as a heterotetrameric assembly. Specifically, the role of the NR2 subunit in epilepsy was evaluated by determining the impact of GPR40 on NMDAR regulation (Figure 2).

In order to study the regulation and cell surface expression of NR2 subunits, the experimenters treated mice with KA and PTZ, then harnessed hippocampal slices from these mouse models. They quantified total expression and cell surface expression of both NR2A and NR2B subunits for the GW9508, GW1100, and DMSO groups and compared them against a standard control of b-actin. It was found that when compared to the DMSO group, NR2A/B showed no significant change in total expression for both the GW9508 and GW1100 models (Figure 2). However, the ratio of surface to total expression of both NR2A and NR2B was significantly reduced for the GW9508 agonist treatment group whereas the opposite results were observed for the GW1100 antagonist treatment group. (Figure 2). These results suggest that GPR40 impacted the cell surface expression of both NMDA subunits in the hippocampal tissues.

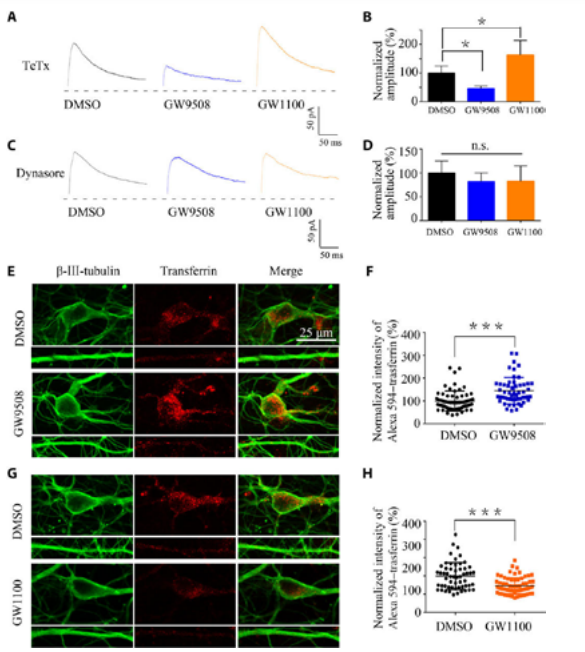

Figure 3. GPR40 Regulates NR2A and NR2B Endocytosis, thereby Affecting NMDA-mediated Postsynaptic Currents. CA1 Hippocampal neurons were isolated from brain tissue treated with endocytosis and exocytosis blockers. a-d) Measured NMDAR-EPSCs after treatment with 0.1 M TeTx or 80 mM dynasore (n=5 in each group). Significant difference (*P < 0.05) observed between control and treatment groups when treated with TeTx. One-way ANOVA and Tukey's Test. e-f) Confocal image and analysis showing differences in Alexa 594-transferrin uptake between 20 M GW9508 and DMSO treatment groups. g-h) Confocal image and analysis showing differences in Alexa 594-transferrin uptake between 20 M GW1100 and DMSO treatment groups (Figure taken from Yang et al., 2018).

This finding was further analyzed, shedding light on the regulation of GPR40 on the surface level expression of NMDARs. This was done through the examination of NMDA-EPSCs (NMDA-excitatory postsynaptic currents) on specific CA1 hippocampal neurons that were obtained from brain tissue slices treated with endocytosis and exocytosis blockers. Interestingly, NMDA-EPSCs amplitudes were decreased in GW9508 but increased in GW1100 (Figure 3B). However, this effect was not seen in the presence of the endocytosis blocker dynasore, suggesting that GPR40 regulates MNDA transmission through endocytic mechanism (Figure 3B, D).

In order to quantify the effect of GRP40 on endocytosis, an Alexa-594-transferrin uptake assay of cultured neurons that had been previously treated with GW9508 and GW1100 was used. GW9508 showed an increase in comparison to DMSO whereas GW1100 showed a decrease in comparison to DMSO, which was seen via the levels of uptake/intensity of the fluorescently conjugated Alexa 594-transferrin (Figure 3F, H). Thus, this confirmed that GPR40 in fact has an important role in the regulation of NMDAR endocytosis.

The molecular mechanism regarding GPR40 regulation of NMDAR-mediated excitatory synaptic transmission has yet to be uncovered, however, this study provided some preliminary support for GPR40 and NMDA receptor interaction. The surface expression of NMDARs was shown to have a critical role in NMDAR-mediated postsynaptic responses, and their mislocalization would therefore explain the possible pathological impact on epilepsy.

An interesting implication from this paper is the role that interactions between proteins had on the surface cell expression of NMDARs. More specifically, the reciprocal co immunoprecipitation, a technique that serves to precipitate a protein antigen out of a solution via the use of a specific protein binding antibody, showed that GPR40 directly interacts with NR2A and NR2B. Additionally, the activation of GPR40 led to decreased binding with NR2A/B, while the inhibition of GPR40 led to increased binding. This supports the idea that GPR40 is therefore involved in the regulation of both neuronal excitability and epileptic activity. The activation of GPR40 is thus thought to decrease epileptic seizures in animal models, as well as NMDAR-mediated postsynaptic transmission, which allowed for a new antiepileptic target to be established.[3] Overall, these results demonstrate that GPR40 activation decreases epileptic seizures through binding to NR2A/NR2B, inducing increased endocytosis of NMDA receptors, and therefore affecting NMDAR-mediated postsynaptic transmission and excitability.

4. Conclusion and Future Directions

Overall, more research is needed to understand the complex interaction between sleep and epilepsy. Sleep is a very important factor to consider in epilepsy patients and epileptic patients need to make sure they have a consistent sleep schedule.

In summary, Yang et al. found that increased GPR40 expression resulted in decreased binding to the NMDA receptor subunit NR2B which resulted in neuroprotective effects. One potential area of future research includes the downstream signaling interactions, as they can provide insight in the development of treatments.[3] In a previous study by Frasca et al., NR2B was studied by pharmacologically blocking NMDA receptors with Ifenprodil.[23] Their data suggests that the reduction of neurodegeneration during epileptogenesis was due to the block of excitotoxicity.[23] The findings of Frasca et al.[23] apply to the research discussed by Yang et al.[3], particularly in reference to the NR2B subunit. While the Yang study observed the binding of GPR40 to NR2B to be a method of NMDA receptor regulation, the Frasca study specifically examined the role of phosphorylation as a form of NR2B and NMDA receptor regulation. These two different approaches towards NMDA receptor regulation could influence future treatment development. The GPR40 and NMDA receptor interactions studied by Yang et al. showed an

increase in GPR40 expression. The phosphorylation of the NR2 subunit of NMDA receptors shown by Frasca et al. resulted in reduced cell death; perhaps an increase in GPR40 could induce the same effects.[3, 14] More research is required to determine if GPR40 is indeed a therapeutic target and can mediate the effects of epilepsy.

Yang et al. established a connection between GPR40 and NMDA receptors, which is a first and necessary step in studying this pathway further. On a gross anatomy scale, expression of GPR40 is localized to the hippocampus and cortex. Additionally, they found that GPR40 expression increased in epileptic brains compared to non-epileptic brains. These findings shed light on the effects of GPR40 on spine density and NMDA signaling amplitude.[3]

All of this further research can significantly contribute to the current understanding of epilepsy. This disease, characterized by the spontaneous recurrence of unprovoked seizures, significantly impacts the daily life of those afflicted with the condition.[1] Furthermore, advancements in the understanding of epilepsy can potentially shed light on the causes of other symptoms of the condition at a molecular level, such as confusion, anxiety, and deja vu.[1] Hopefully, the findings from Yang et al., along with future research on synaptic transmission in this field, can help push the scientific community one step closer to finding a treatment for this disorder.

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The Burn Behind the Bullet: Understanding Black Mothers' Experiences After Losing a Child to Gun Violence in Washington, DC-Baltimore City Metropolitan Region

Denzell Brown

The Burn Behind the Bullet: Understanding Black Mothers' Experiences After Losing a Child to Gun Violence in Washington, DC-Baltimore City Metropolitan Region

Denzell Brown

Department of Psychology and African American Studies, Georgetown University, Washington D.C.
E-mail: dob15@georgetown.edu
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Abstract

The purpose of this research article is to examine how complicated grief, post-traumatic stress, and depressive symptoms induced from losing a child to gun violence affect traits of resilience and post-traumatic growth among a sample of Black mothers living in Washington DC and Baltimore, Maryland. This research project was executed by surveying 4 Black mothers who lost a child to gun violence *(B.M.C.G.V.)* that resided in the Baltimore-Washington area to assess grief, traumatic stress, and depression. Participants also completed an oral interview that focused on resilience, post-traumatic growth, and policy recommendations. Findings associated with post-traumatic stress indicated that all Black mothers in this study reported it was somewhat true that they avoid things that remind them of their loved ones (n= 4, 100%), and 3 out of 4 of the mothers felt cut off or distant from other people since their loved one died (n= 3, 75%). Outcomes related to complicated grief revealed that all mothers in this study reported that they felt a great deal of loneliness since their child had died (n= 4, 100%). Moreover, 3 out 4 *B.M.C.G.V.* reported that memories of their child made them upset in the last past 7 days (n= 3, 75%). Results aligning with post-traumatic growth displayed that all Black mothers in this study reported it was mostly true that they learned they were stronger than they originally thought they were after losing a child to gun violence (n=4, 100%). Additionally, 3 out of 4 Black mothers in this study stated that it is mostly true that they developed a strong religious faith upon losing a child to gun violence (n= 3, 75%). Furthermore, 3 out of 4 Black mothers in this study reported that they found a stronger sense of purpose in life upon losing a child to gun violence (n = 3, 75%). Findings related to depressive symptomatology contained a large amount of variation and did not produce any significant results. The data results from the oral interview indicated that 9 common characteristics emerged from Black mothers who lost a child to gun violence in this study which included Black mothers explaining their character traits as *Loving, Committed,* and *Strong*. Subsequently, Black mothers classified their coping strategies as *Active Coping (Embracing Self-love, Forgiveness, and Faith in God)* and *Avoidant Coping (Denial, Betrayal, and Not Coping)*. Lastly, Black mothers' policy recommendations in this study focused on themes such as *Demanding resources and Laws on gun violence prevention.*

Keywords: complicated grief, post-traumatic growth, gun violence

1. Introduction

Throughout the United States of America, a disproportionate number of Black youths become homicide victims of gun violence annually, and every year Black mothers nationally have to deal with the psychological consequences of the death of their own child. To date, much of the research on gun violence in predominantly Black communities tend to focus on African American male-identified youth. Yet, there is a large amount of literature that indicates *B.M.C.G.V.* experiences compounding levels of trauma, complicated grief, and depression. For instance, Black mothers experience various incarnations and manifestations of racism and sexism.[1] Given that Black mothers' experiences with gun violence are impacted by racial-gendered stereotypes, African American mothers are also consequently labeled as being "strong Black women." The discourse of being "strong" tends to normalize distress inducing levels of selflessness and powerlessness among African-American mothers.[2] Therefore, Black mothers face the multiplying effects of racism and sexism while simultaneously experiencing forms of complicated maternal grief that emerge from losing a child to gun violence. Maternal grief is considered to be the most persistent and profound grief.[3] In the process of Black mothers navigating maternal grief, African American mothers may also feel a sense of injustice associated with their child's death, as well as experience symptoms of complicated grief due to multiple past and present traumas.[4] As shown, prior research suggests that Black mothers who lose children to gun violence experience post-traumatic stress, complicated grief, and depressive symptomatology.

On a spectrum, Blacks mothers encounter multi-layered levels of psychological stress that derive from losing a child to both civilian and police gun violence. Considering that a large number of Black mothers lose children to police gun violence, African American mothers of color also often feel betrayed by law enforcement and the criminal justice system.[4] Furthermore, African American mothers are also often traumatized daily by the sudden loss of their loved ones and re-traumatized by the news of another murder in the country.[4] As a protective barrier to cope with the complicated grief that absolves from losing a child to gun violence, Black mothers appear to demonstrate a sense of resilience in childbearing after the loss.[4] Black mothers tend to embody resilience by relying on solidarity and collective survival through community mothering practices that have been characterized as Black 'activist mothering'.[5] The practice of Black 'activist mothering' allows for African American mothers who lost a child to gun violence to manage their experiences with complicated grief by having a public space to share grief and conceptualize healing.[5] In cases where Black mothers pursue a journey of healing, some Black mothers embrace post-traumatic growth as a method to augment the traumatic stress associated with losing a child to gun violence. As referenced in a recent mixed-methods study on Black low-income mothers who survived Hurricane Katrina, catastrophic losses in some cases create spaces for post-traumatic growth for survivors.[6] Therefore, the goal of this research paper will be situated on illustrating *B.M.C.G.V.* coping mechanisms, healing methods, and post-traumatic growth qualities.

1.1 Depressive Symptomatology

Depressive symptomatology can be expressed through a range of different emotions including constant feelings of sadness, emptiness, restlessness, guilt, and social isolation.[7] According to King-Hannays et al 2013, Black mothers' bereavement experiences upon losing a child to gun violence often impact this population's ability to maintain social relationships. The results of this study revealed that there was an association between Black mothers whose children were homicide victims and mothers who experienced altered relationships within their family-friend networks (directly correlated with feelings of isolation and sadness).[8] The psychological distress that Black mothers who have lost a child to gun violence endure can be characterized by intrusive thoughts about the deceased, a subjective sense of numbness, detachment or absence of emotional

responsiveness, searching for the deceased, and loneliness as a result of the death.[4]

1.2 Post-Traumatic Stress

Post-traumatic stress can be characterized by the avoidance of thoughts and activities associated with traumatic events and can result in a constant state of hypervigilance.[9] The experience of losing a child to gun violence is a traumatic event that can result in a variety of distressful emotions such as meaningless and devastation which can lead to debilitating traumatic stress.[10] Research on post-traumatic stress identifies the death of a child as the most severe stress encounter for parents.[11] Specifically for Black mothers who lost a child to gun violence, death-related traumas were an ongoing source of fear and pain.[4] The ongoing fear and pain that Black mothers experience are related to grief and rumination about the causes and consequences of the loss.[12]

1.3 Complicated Grief

Complicated grief may include symptoms like severe pangs of emotions, denial of implications of the loss to the self, and neglect of necessary adaptive activities.[13] Grieving a violent death is different from a normal or uncomplicated bereavement behavior as the difference in the death is caused by human intent or negligence.[10] Therefore, disturbed or complicated grief can be understood as a condition that arises when the normal progression of grief does not occur.[13] Due to the life-altering experience of losing a child to gun violence, Black mothers experience prolonged feelings of pain and detachment from others which ultimately heightens the emotional presence of complicated grief. There is a direct correlation between *B.M.C.G.V.* and Black mothers' reported experiences of complicated grief. For instance, according to a recent study by Burke et al 2010, which investigated African Americans' processing of homicide bereavement in Memphis Tennessee, findings reveal that Black mothers reported bereavement outcomes such as complicated grief, PTSD, and depression.[15] The complicated grief, post-traumatic stress, and depressive symptoms that *B.M.C.G.V.*, also often encourage Black's mother's to independently search for effective ways and alternative strategies to understand the death of their child. Furthermore, prior research suggests that throughout the process of navigating effective ways to understand their children's death, that Black mothers also demonstrate resilience and self-coping strategies.

1.4 Resilience

Black mothers that are grieving the death of their children often develop resilience and coping strategies by relying on social networks and appraising positive meaning to the occurrence of death. Based on the Bailey et al 2013 study, which examined *B.M.C.G.V.* in Canada, social support and assigning a positive meaning to the death of one's child mediated the effects of post-traumatic stress that Canadian Black mothers experienced. Social support and positive appraisal as mediating factors ultimately played a role in increasing feelings of resilience among Canadian Black mothers within this sample that lost children to gun violence.[11] This specific finding highlights how Black mothers that lose children to gun violence do not only experience depression, grief, and trauma, but also that they embody resilience and embrace self-coping mechanisms despite their traumatic experiences to affirm emotional sustainability.

1.5 Post-Traumatic Growth

Post-traumatic growth (PTG) consists of positive psychological changes that arise from experiencing new opportunities that come to light as a result of a traumatic experience, as well as through the cognitive and emotional processing of trauma-related thoughts, sensations, emotions and, memories.[16, 17] Notably, multiple findings demonstrate that people identifying with oppressed identities – such as racial minorities or women – report higher PTG than those identifying with more privileged statuses. Given that Black mothers identify as a racial minority and are normatively classified as women, post-traumatic growth is a relational variable that

applies to African American mothers who lost a child to gun violence. Research on the intersection of post-traumatic growth and gun violence in the Baltimore-Washington area claims that post-traumatic growth can be conceptualized as, how individuals work to reconcile their trauma with their religious beliefs and can strengthen those beliefs, lead to positive spiritual change, shifting priorities, and an appreciation for life post-trauma.[18] This classification of post-traumatic growth will serve as the working definition used to explain Black mothers' post-traumatic experiences after losing a child to gun violence in this article. In the context of the Baltimore-Washington DC region, some Black mothers' process of healing is marked by engagement with post-traumatic growth as means to cope with the trauma associated with the death of their child.

As shown, there is evidence throughout inner cities in the U.S and Canada that Black mothers experience psychological depression, post-traumatic stress, and complicated grief while mourning the death of their children who were homicide victims of gun violence. However, what has not been clarified in this area of research are the specific and intricate ways in which Black mothers develop resilience, coping mechanisms, and post-traumatic growth qualities in the process of grieving the death of their child. The specific ways that Black mothers develop resilience, embrace post-traumatic growth, and cope with the death of their children who are homicide victims has not been researched in-depth on a mass scale in the field of psychology, rendering their experiences invisible. Research surrounding this topic tends to solely focus on Black mothers' grief, depression, and psychological trauma in both of these major cities. While this is important, it leaves out possible moderating factors like resilience, coping, and post-traumatic growth that also characterizes Black mothers' lived experiences which, if identified, could provide policymakers and practitioners with levers for improving mental health services within Black communities throughout the Washington, DC, and Baltimore City metropolitan region.

1.6 Current Study

This study will seek to investigate Black mothers whose children have been homicide victims of gun violence throughout the Baltimore-Washington DC regions' emotional experiences of depression, complicated grief, and posttraumatic stress. Additionally, this study will also document these women's processes of formulating resilience, post-traumatic growth, and effective coping strategies. This research project aims to not only analyze the complicated grief, psychological depression, and posttraumatic stress that Black mothers face whose children were victims of gun violence but also to document Black mothers' independent self-coping strategies, methods of resiliency, and post-traumatic growth capabilities. Findings from this line of research will provide actionable steps for local policymakers and practitioners (social workers; community workers) to support the needs of Black mothers enduring the death of a child due to gun violence in the Baltimore- Washington, DC region.

2. Methods

2.1 Research Design

This study used a mixed-methods descriptive research design to examine how grief, post-traumatic stress, and depressive symptoms that are induced from losing a child to gun violence affect the variables of resilience and posttraumatic growth among a sample of Black mothers living in the Washington DC-Baltimore metropolitan area.

2.2 Research Questions

This study sought to examine three specific research questions which includes:

(1) How do Black mothers that have children who have been homicide victims of gun violence experience feelings of depression, grief, and post-traumatic stress?

(2) Do Black mothers who lose a child to gun violence embody characteristics that resemble variables of post-traumatic growth?

(3) What coping strategies and forms of resilience do these mothers use or demonstrate that may be effective in mitigating the crippling effects of losing a child to violence?

2.3 Hypotheses

(1) Black Mothers in Washington DC who have lost a child to gun violence will exhibit emotional expressions of trauma, grief, and depression. (Independent variable = gun violence; dependent variable = trauma, grief, depression)
(2) Black Mothers in this study will showcase high levels of self-sufficient coping mechanisms, post-traumatic qualities, and traits of resilience

2.4 Sample

The sample of this study includes a total of 4 Black mothers that are between the ages of 18-65 years old. All of the women involved in this study met the eligibility criteria of losing a child to gun violence and living in the Washington-DC and Baltimore city metropolitan area. To limit the possibility of emotional harm, participation in this study was restricted to only Black mothers in the Washington-Baltimore area who lost a child to gun violence 1 year or more prior to the start date of the study in December 2020. Demographic data in Table 1 shows Black mothers that participated in this study's occupation status and economic welfare dependence on public assistance. Furthermore, Table 1 also indicates the context under which the gun violence occurred, including the average age of the child when they passed away, if the individual(s) responsible for this death were identified, and whether the individual(s) responsible were law enforcement agents (Table 1).

Table 1: Participant child's age at death, knowledge of perpetrator(s), perpetrator(s) involvement with law enforcement, marital status, and dependence on welfare assistance

Participant	Child Age at death	Does the participant know the individual responsible for the death of their child?	Were individuals responsible for the child death police officers or law enforcement agent(s)?	Marital Status	Public Assistance food stamps (SNAP), Medicaid, housing assistance, welfare (TANF), etc?
Participant 1	*17*	*Yes*	*Yes*	*Widowed*	*No*
Participant 2	*14*	*Yes*	*No*	*Married*	*No*
Participant 3	*24*	*Yes*	*Yes*	*Single*	*No*
Participant 4	*29*	*Yes*	*No*	*Single*	*No*

2.5 Recruitment and Data Collection

Upon receiving approval from Georgetown University's Institutional review board, a multiplicity-network sampling method was used to recruit participants from several non-profit organizations and community grief support groups. Multiplicity or network sampling is a process by which individuals distribute recruiting information with neighbors, co-workers, and members of their community at large. Multiplicity sampling is best used for locating and measuring rare populations.[18] Using this sampling method, this study began with a target population of 2 Black mothers who completed the survey and interview portion of this research design after being referred by community mental healthcare providers and non-profit organizations leaders. The individuals and organizations featured in the initial target population of this study then shared recruiting materials such as flyers and the principal investigator's contact information with other women who met the eligibility criteria to participate in this research design. Given the grief-provoking nature of the content discussed during this study, all participants were provided with a phone number of a licensed grief therapist that specializes in trauma-informed violence interventions. Lastly, all participants were also provided with access to Washington DC's

Department of Behavioral services mental health mobile hotline and Baltimore city's accredited crisis center's phone line.

2.6 Measures

To assess the demographic data of the population of Black mothers involved in this study participants were asked a series of self-reported survey questions regarding marital status, occupation, and public assistance income benefits. Marital status was assessed among participants by asking the question "What *is your current marital status?*" Participants occupation was investigated using the question *"Are you currently working for pay, full or part-time?"*. Income was determined by asking about participants' access to receiving public benefits using the question "Do you receive any public benefits like food stamps (SNAP), Medicaid, housing assistance, welfare (TANF), etc?" Lastly, to determine the context and occurrence of this sample of Black mothers' children's death, participants were asked about the age of the child at death, the individual(s) responsible for the death, and if this or these individual(s) were law enforcement agents. The questions used to obtain this information included "Please enter the age of your child or loved one were when they passed away", "Do you know the individual(s) who was responsible for the death of your child or loved one?", "Were these or these individuals police officer(s) or member(s) of law enforcement agencies?" (Table 1).

2.6.1 Complicated Grief (9 Items)

Questions measuring complicated grief among *B.M.C.G.V.* were drawn from the Prigerson, et al. 1995 Inventory of Complicated Grief (ICG) which measures feelings of sadness, anger, and disbelief on a 4-point Likert scale (0=never,1=rarely, 3=sometimes, 5=Often).[19] The inventory of complicated grief asks questions such as "how often do you feel empty without the person who died?" and "how often do you feel bitter over this person's death?". The ICG's internal consistency, as reported by Prigerson, et al. (1995), was very good; the alpha coefficient was .94. The test-retest reliability was found in the same study to be .80. In addition, this scale has a well-validated clinical cut point.[20, 19]

2.6.1 Post-Traumatic Stress (5 Items)

To document the post-traumatic stress that *B.M.C.G.V.* experienced this study used the Daniel S. Weiss. Revised Trauma Impact Event Scale – which captures the amount of stress that individuals associate with traumatic events on a 3-point Likert scale (1=somewhat true, 2 = mostly true, 3 = Very much true.) Items on the Impact-Event scale seek to capture characteristics of intrusion and avoid avoidance.[21] Sample questions from this scale include: for example, "How much trouble are you having/have you had accepting the death of ____?) and (ex: "How much do you avoid things that remind you of your loved one?). The internal consistency of intrusion and avoidance on Daniel S. Weiss Revised 2007 event scale was acceptable (Cronbach's, alpha for intrusion = 0.79, for avoidance = 0.82). The test–retest reliability was satisfactory, with coefficients of 0.87 for intrusion and 0.79 for avoidance.[21]

2.6.2 Depressive Symptomatology (1 Item)

One item was used to assess depressive symptomatology present among *B.M.C.G.V.* This was a specific yes or no depression screening question that asked, "during the last month have you had a period of 2 weeks or more when nearly every day you felt sad, empty, or depressed for most of the day or you lost interest in most things like work, hobbies, and other things you usually enjoy?". This question was derived from Georgetown University's Professor Dr. Anna D. Johnson prior research on depression among parents.

2.6.3 Post-Traumatic Growth (4 Items)

To record post-traumatic growth among *B.M.C.G.V.* in this study use Tedeschi & Calhoun 1996 post-traumatic growth inventory. The Posttraumatic Growth Inventory measures

positive outcomes reported by individuals who have undergone a traumatic event on a 4-point Likert scale (1= Not true about me, 2= A little true, 3 = Somewhat true, 4= Mostly True). This scale includes items such as, "I have a stronger sense of religious faith, I discovered I am stronger than I thought I was, etc". The internal consistency of the resulting 21-item Post-traumatic growth inventory is a = .90. The test-retest reliability for the 21-item FTGI was acceptable at r = .71.[16]

2.6.4 Resilience and Coping Strategies (3 Items)

Using a qualitative interview approach, this study interpreted the resilience of *B.M.C.G.V.* and their self-coping strategies through a narrative analysis methodology with a topical story focus. A narrative analysis helps focus on the events and experiences that shape a person's self-understanding.[22] Topical stories are about a particular event or specific character.[23] In the context of this study, Black mothers were centered in the analysis and the event of losing one child to gun violence was captured as the particular event. Throughout an oral interview, Black mothers were asked 3 specific questions which include "1. If you could describe yourself as a mother in one word, what would it be? 2. How did you cope, understand, or make meaning of the death of your child? 3. Taking into account that you endured the experience of losing a child to gun violence, what political policy(s) would you like to see reflected to possibly aid yourself or anyone else in this position?". These questions were used to situate Black mothers at the forefront of documenting their own experiences considering that narratives are extremely helpful in gaining an understanding complex topics about which little is known.[24]

2.7 Procedure

Participants who met the inclusion criteria of being a Black mother who lost a child to gun violence in the Baltimore-Washington area 1 year or more prior to the start date of the study in December 2020 were scheduled to complete the survey and interview portion of this study. Prior to beginning the study, Black mothers were given a detailed-written summary explaining the purpose of the research project along with an informed consent signature component that asked for participants' permission to participate in the study. Given the COVID-19 pandemic restrictions, Black mothers that agreed to participate in this study were given the consent form and online survey information via email or text and completed the survey via zoom video call or telephone. To control for social desirability bias reporting among participants before completing the self-reported survey the principal investigator re-assured participants that all survey results will be completely confidential and free of judgement. Additionally, participants were not informed or notified of which survey items corresponded to the topics being evaluated such as post-traumatic stress, complicated grief, or depression in order to further reduce the likelihood of reporting bias and experimenter effects. As participants began the survey, they were notified that their responses will be completely anonymous and no identifying information would be collected such as (i.e. last names, addresses, birthdates, etc.). Participants completed the online self-reported questionnaire survey with their cameras and microphones off and were informed if they had any questions that participants may unmute themselves to ask the principal investigator for assistance.

Upon completing the survey, participants were then welcomed to engage in an oral interview with the principal investigator. To ensure safety measures in the light of social distancing guidelines, participants completed the oral interviews via phone call or using the online zoom video platform. The oral interview began with a Greeting and Brief introduction of the Principal Investigator's name and reason for doing the research. The principal investigator then proceeded to inform the participants of their rights to confidentiality, described how the interviews will not be recorded, and explained that if at any

time mothers felt uncomfortable or were unwilling to answer any interview question, the participant may choose not to answer questions or discontinue their participation in the study. Participants were then guided into a narrative discussion focusing on the 3 qualitative items and prompted to take as much time as needed to assess and fully answer each question contingent upon their comfortability. After participants completed the oral interview, each participant was thanked for their participation and offered a complimentary $30 Safeway gift card.

2.8 Analytic Strategy

Given the small sample size of this study, the survey data were analyzed using basic descriptive statistics. Therefore, the most frequently reported information that Black mothers selected on the self-reported measure were organized by total and calculated into percentage values. The use of percentage values was the most cohesive and efficient quantitative analysis that can be used to showcase the data outcomes of *B.M.C.G.V.* reported experiences with post-traumatic stress, complicated grief, depressive symptomatology, and post-traumatic growth.

The oral interviews were analyzed and transcribed using a qualitative inductive thematic analysis. An applied inductive thematic analysis is a process that includes comparing and analyzing concepts found within participants' narratives, for the purpose of identifying central themes that helped to explain grief and coping strategies.[4] The raw transcript data was imported into the Dedoose qualitative-mixed methods software and then analyzed by identifying emerging themes from participants' responses. Table 2 summarizes the responses participants provided to the qualitative oral interview items and the themes/sub-themes that emerged in the data.

3. Results

The results from the inductive thematic analysis indicated that 9 themes emerged from *B.M.C.G.V.* oral interviews. These 9 themes will be listed and discussed in the next few sentences. For qualitative item 1 which assessed Black mothers character traits, themes such as *Loving, Committed,* and *Strong* were prevalent. On qualitative item 2 which documented Black mothers' experiences with coping and resilience, the themes of: *Active Coping (Self-love, Forgiveness, Faith in God)* and *Avoidant Coping (Denial, Betrayal, and Not Coping)* were shown directly. Lastly, qualitative item 3 that focused on Black mothers' policy recommendations themes like *Demanding Resources for Families and Laws on Gun Violence Prevention that give attention to the victims* emerged from the data (Table 2).

Table 2: Qualitative themes of resilience and coping strategies.

Question	Theme	Direct Quote
Q1: *If you could describe yourself as a mother in one word, what would it be?*	Loving	***Participant 1:*** **"Loving"** ***Participant 4:*** "Unconditionally **loving"**
	Committed	***Participant 2:*** **"Committed"**
	Strong	***Participant 3:*** **"Strong"**
Q2: *How did you cope, understand, or make meaning of the death of your child?*	Active Coping *Self-love* *Forgiveness* *Faith in God*	 **Participant 1:** "embracing **self-love** and self-care using meditation/yoga" **Participant 1:** "learning to pursue **forgiveness** to turn purpose into" **Participant 3:** "greater **faith in God"**
	Avoidance coping *Denial* *Betrayal* *Lack of coping*	 **Participant 4:** "felt **denial** things were in slow motion" **Participant 1:** "initially feeling of **betrayal"** **Participant 2:** "I **did not cope"**
	Laws on gun violence prevention and centered attention on victims	**Participant 4:** "**prevent guns** from getting on the streets" **Participant 2:** "**gun laws** to become more appropriate" **Participant 3:** "**more attention** to cases that are not high profile" **Participant 4:** "a space where children in elementary school learn coping mechanisms and **alternatives to gun violence"**
Q3: *What political policy(s) would you like to see reflected to possibly aid yourself or anyone else in this position?*	Demanding resources for families	**Participant 1: Implementing resources** that encourage and **make court testimonies for mothers and families who have been impacted by gun violence more efficient**." **Participant 2:** "**More resources** to the court system and the victims' families. " **Participant 3:** "**More resources** for the family for the families when child lost from law enforcements, more forensics test, free lawyers, and **mental health resources for the fathers"**
	Laws on gun violence prevention and centered attention on victims	**Participant 4:** "**prevent guns** from getting on the streets" **Participant 2:** "**gun laws** to become more appropriate" **Participant 3:** "**more attention** to cases that are not high profile" **Participant 4:** "a space where children in elementary school learn coping mechanisms and **alternatives to gun violence"**

The quantitative results obtained from the self-reported measures demonstrated that *B.M.C.G.V.* experience compounding levels of post-traumatic stress, complicated grief, and post-traumatic growth.

3.1 Post-Traumatic Stress

Findings associated with post-traumatic stress indicated that all Black mothers who lost a child to gun violence in this study reported that it was somewhat true that they avoid things that remind them of their loved ones such as going places they used to go together and looking at their pictures. (n =4, 100%). 3 out of 4 of the mothers in this study also reported that it was somewhat true they felt cut off or distant from other people since their loved one died – specifically people they used to feel close to such as their family and friends (n=3, 75%).

3.2 Complicated Grief

Outcomes related to complicated grief revealed that all mothers reported that they felt a great deal of loneliness since their child has died. (n= 4, 100%). Furthermore, 3 out 4 *B.M.C.G.V.* reported that memories of their child upset them in the last past 7 days (n= 3, 75%).

3.3 Post-Traumatic Growth

Results aligning with post-traumatic growth displayed that all Black mothers in this study reported it was mostly true that they learned they were stronger than they originally thought they were after losing a child to gun violence. (n=4, 100%). 3 out of 4 Black mothers in this study stated it is mostly true that they developed a strong religious faith upon losing a child to gun violence. (n= 3, 75%). Additionally, 3 out of 4 Black mothers in this study reported that they found a stronger sense of purpose in life upon losing a child to gun violence (n = 3, 75%).

3.4 Depressive Symptomatology

Black mothers' reports of depressive symptomatology contained a large amount of variation and did not produce any significant results.

4. Discussion – Policy Recommendations

The findings of this study that captured post-traumatic stress, complicated grief, and posttraumatic growth among *B.M.C.G.V.* are consistent with pre-existing literature and hypothesis 1. Hypothesis 1 indicated that Black Mothers in the Washington DC- Baltimore area who have lost a child to gun violence will exhibit emotional expressions of trauma, grief, and depression. As shown, in the quantitative and qualitative results of this study: the homicidal loss of a child is one of the most disruptive psychological traumas that a mother can experience.[25] Post-traumatic stress can be characterized as an experience of distressing oscillation between intrusion and avoidance.[21] Black mothers reported experiences of avoiding things that remind them of their child is a detailed indicator that exemplifies the post-traumatic stress associated with losing a child to gun violence. Moreover, the post-traumatic stress that *B.M.C.G.V.* in this study experienced was further marked by feeling distant from their close family members because of the intrusive emotions that these mothers encountered when thinking about their children as homicide victims. Due to the type of death—homicide—which entails suddenness, lack of anticipation, violence, and destruction, a longer grieving interval may be present among Black mothers who lost a child to gun violence.[4] It is important to note that all *B.M.C.G.V.* included in this study lost their child 1-3 years more prior to the start date of this study. Despite this interval of time, all Black mothers in the study reported they experienced constant feelings of loneliness and that memories of their child in the past 7 days upset them. In the midst of these Black mothers

processing the complicated grief associated with losing a child, some Black mothers sought to reconcile their trauma with their religious beliefs which can strengthen those beliefs, lead to positive spiritual change, shifted priorities, and an appreciation for life post-trauma.[16] Hypothesis 2 which predicted that Black Mothers demonstrate high levels of self-sufficient coping mechanisms, post-traumatic growth qualities, and traits of resilience was proven to be true. Post-traumatic growth was a prevalent variable and resilient coping mechanism that allowed mothers to manage the stress associated with the loss. As shown in this study, 3 out 4 of Black mothers reported that it was mostly true that they developed a strong religious faith and found a stronger sense of purpose in life upon losing a child to gun violence. Most notably, all of the mothers in this study reported it was mostly true that they learned they were stronger than they originally thought they were after losing a child to gun violence. Therefore, Black mothers' reported experiences of post-traumatic growth in this study can be seen as a positive psychological change due to the experience and processing of the disaster and its aftermath.[26]

The qualitative findings demonstrated that there is a multitude of resilience methods and coping strategies that shape *B.M.C.G.V.* healing processes. Based on the reported data in this study, Black mothers explained themselves as *loving, committed, strong, and unconditionally loving* despite their lived experiences of losing a child to gun violence. *(See Table 2)*. This initial finding encouraged Black mothers to speak toward their experiences with coping. Coping is the set of intentional, goal-directed efforts people engage in to minimize the physical, psychological, or social harm of an event or situation.[27, 28] Black mothers reported coping experiences diverged among two outcomes which were active coping and avoidant coping. Active coping refers to the utilization of psychological or behavioral coping efforts that are characterized by an attempt to use one's resources to deal with a problem situation.[29] These responses are designed either to change the nature of the stressful situation or event in order to decrease the problematic nature of that situation or event, or to modify how one thinks and feels about it in order to change one's reactions to it.[29] Avoidance coping involves cognitive and behavioral efforts oriented toward denying, minimizing, or otherwise avoiding dealing directly with stressful demands.[30] Black mothers reported active coping styles were displayed through themes such as *self-love, forgiveness, and faith in god*. These active coping strategies were used by Black mothers as a leveraging effort to reframe and manage stressful emotions surrounding losing a child to gun violence. On the contrary, Black mothers' avoidant coping styles in this study were situated on *denial, betrayal, and lack of coping*. The avoidant coping style's mothers demonstrated were centered on focal points that allowed mothers to dissuade and desist from coping as a way to sustain their psychological distress surrounding losing a child to gun violence. Both of the coping styles mothers demonstrated were effective variables that formulated the policy suggestions that *B.M.C.G.V.* in this study recommended.

Outcomes regarding Black mothers' policy suggestions can be interpreted through a Black feminist pedagogical lens. A Black feminist pedagogy is a methodology for promoting equality and multiple visions and perspectives that parallel Black women's attempts to be and become recognized as human beings and citizens rather than objects and victims.[31] *B.M.C.G.V.* in this study's policy recommendations were centered on mothers advocating for equity and restorative laws that valued their experiences as citizens who lost a child to gun violence. Black mothers reported themes detailing that Black mothers were: *Demanding resources for families* and *Laws on gun violence prevention that are centered around victims*. The policy recommendations that Black mothers

in this study provided focusing on garnering *more resources to families in the court of law*, *providing free legal services,* and *implementing alternatives to gun violence* can be viewed as progressive solutions for change that can be adopted by council members and mental health providers in the Baltimore-Washington area.

Taking this into account, the next steps should focus on analyzing how the deliverable policy outcomes that Black mothers reported relating to legal advocacy support and mental health pipelines can be explored as effective buffers to augment the emotional burden of losing a child to gun violence. Furthermore, future research should study post-traumatic growth, complicated grief, post-traumatic stress, and depressive symptoms among *B.M.C.G.V.* with a larger sample size in order to gain statistically significant results. Lastly, the field of psychology should seek to use intersectional tailored psychometric scales that are attentive to *B.M.C.G.V.* social identities and lived experiences in order to gain rich and meaningful data on this topic.

5. Limitations

This study includes several limitations that should be addressed in future research. For example, given the small sample size of *B.M.C.G.V.* that were involved in this study, findings should be interpreted with caution. This small sample is not fully representative of the larger population of *B.M.C.G.V.* in the Baltimore-Washington area therefore the outcomes in this study are not generalizable. Moreover, considering that this study does not include a comparison group to *B.M.C.G.V*, the findings of this study do not control for external factors that may have also influenced this population's reported outcomes relating to depression, grief, and post-traumatic stress. For example, the high variability within the marital status among *B.M.C.G.V* in this study may have impacted each mothers' reported outcomes regarding depression, complicated grief, and post-traumatic growth. Additionally, this study was conducted in the midst of the COVID-19 pandemic mothers reported feelings of complicated grief, post-traumatic stress and depressive symptoms may have been heightened due to the global crisis that was occurring during the time this data was assessed.

6. Conclusions

This study examined how post-traumatic stress, complicated grief, and depressive symptoms induced from losing a child to gun violence affect Black mothers' methods of resilience, coping strategies, and post-traumatic growth possibilities. Black mothers' experiences with resilience, coping, and posttraumatic growth is an understudied topic that needs to be more thoroughly investigated, understood, and acknowledged as a notable source of scientific inquiry. Evaluating Black mothers' experiential knowledge with losing a child to gun violence is a necessary topic to research considering that the reported outcomes from this population can allow the field of psychology to understand Black motherhood through an informative lens that showcases an array of alternative healing methods. Black mothers' informative and elusive healing methods can serve as vectors of knowledge that can inform legislation surrounding allocating resources to communities in which Black mothers experience the most prevalence of gun violence. By centering on Black mothers' healing experiences, this study aims to leverage these mothers' socially innovative suggestions to encourage clinicians and councilmembers to improve mental health services for the *B.M.C.G.V.* in the Washington, DC-Baltimore city metropolitan region

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Cancer Models to Defeat Therapy Resistance in Pancreatic Ductal Adenocarcinoma

Britney He

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Britney He

Department of Oncology, Georgetown University Medical Center, Washington D.C.
E-mail: bkh40@georgetown.edu
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Abstract

One of the largest hurdles to the efficacy of cancer therapeutics, and a main cause of relapse, is therapy resistance. In response, researchers have developed model systems to better understand therapy resistance. Cancer research employs several model systems that reflect the biology of actual human tumors: in vitro models (2D, 3D cell cultures), in vivo models (PDX, GEMMS, transgenic), proteomic models, and computational or mathematical models. One cancer that has been extensively modeled is pancreatic ductal adenocarcinoma (PDAC). PDAC is the third most common cause of annual cancer deaths in developed countries; as its incidence and mortality rates continue to increase, PDAC is projected to be the second leading cause of cancer deaths by 2030. Although chemotherapy is a pillar of clinical PDAC treatment, its outcome typically leads to multi-drug resistance, drastically restricting the curative effect of drugs for a variety of tumors. Elucidating the underlying mechanisms for resistance through different models is essential for the development of new strategies and therapies. This review provides insight into the range of *in vitro* and *in vivo* models of pancreatic cancer used in preclinical research. This paper provides an overview of platforms for cancer research with a focus on those devoted to resistance mechanisms in PDAC and to the primary therapeutic intervention for PDAC, gemcitabine (GEM).

Keywords: therapy resistance, pancreatic cancer, in vivo models, in vitro models, computational, proteomic, gemcitabine, PDAC

1. Introduction

One of the most studied cancers is pancreatic ductal adenocarcinoma (PDAC) due to its high stemness and tumorigenicity, making it a major health concern that warrants greater efforts towards earlier detection and improved treatment. There are several models for PDAC on cancer resistance due to the extremely deadly nature of PDAC. Some of the newest and acclaimed models are for PDAC cells. This review will highlight the models used for examining drug resistance in PDAC.

PDAC is the third most common cause of annual cancer deaths in developed countries, and the incidence and mortality rates of this disease are climbing. This disease is projected to be the second leading cause of cancer deaths by 2030.[67] Despite increased survival resulting from various multidisciplinary curative and palliative treatment options, including surgical removal, stent placement, and nonsteroidal anti-inflammatory drugs, the disease outcome is most often fatal. The most recent advances in chemotherapeutic treatment extend the average survival to 8.5-9.4 months.[68,69] Currently, the 5-year survival rate for PDAC for all stages is the lowest among all the cancers at 9%, which drops to 3% for patients diagnosed with distant or metastatic PDAC.[70] The lack of effective treatment options for PDAC contributes to the low survival rate, pointing to the

need for improved treatment and earlier detection.[67]

Medical and surgical treatments for this highly lethal disease are limited and often ineffective. For patients with unresectable PDAC, therapy is restricted to chemotherapy, for which most eventually develop resistance. Gemcitabine (GEM) emerged in 1997 as an alternative for 5-Fluorouracil and ultimately improved survival by a few weeks. The introduction of the FOLFIRINOX treatment scheme (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan) also contributed to a small improvement in survival for patients with an advantaged stage of the disease.[55] Currently, the most effective and tolerated drug is nab-paclitaxel (n-PTX) and, when used with GEM, has modestly prolonged median overall survival.[54,69]

The poor clinical situation and the fact that only three improvements have been introduced over the last 20 years underscore how effective therapeutic strategies for patients with PDAC have been difficult to identify. Despite numerous preclinical investigations and clinical trials, only moderate progress has been made in improving therapeutic strategies.[55] Thus, there is a desperate need for novel drugs, improved radiation protocols, and increased avenues for second- and third-line therapies.[55]

Although chemotherapy is one of the pillars of clinical cancer treatment, its outcome typically leads to multidrug resistance, drastically restricting the curative effect of drugs for a variety of tumors, such as in pancreatic cancer patients.[7,71] Many cancer types that are initially susceptible to treatment often develop therapeutic resistance over the course of the therapeutic regimen. Resistance is due to several intracellular factors, including genetic and epigenetic changes in signaling pathways, drug-metabolizing enzymes, and drug efflux pump mechanisms.[7,71] Pancreatic tumors are especially characterized by genetic instability, intra-tumoral heterogeneity, and distinct desmoplastic stroma that makes it difficult to effectively develop therapeutic strategies for PDAC. The lack of innovative approaches to treating PDAC stems from the high degree of heterogeneity of this tumor with several different histopathological subtypes and limited knowledge on the molecular mechanisms behind tumor development and progression.

The development of chemotherapeutic resistance in cancer patients poses a major clinical problem for chemotherapeutic treatment. The elucidation of underlying mechanisms for resistance through different models is essential for the development of new strategies and therapies.[7,71] Therapeutic resistance in PDAC has been explored through various cell culture and animal model systems. Cell cultures include two-dimensional (2D) culture conditions and, most notably, three-dimensional (3D) culture strategies, such as organoids and spheroids.[36] 3D culture models have incorporated pancreatic stellate cells (PSC) to investigate the prominent desmoplastic/stromal reaction in PDAC.[62] Moreover, cells grown in 3D models showed resistance to GEM and n-PTX, drugs frequently used for PDAC treatment.[36,62] Animal model systems include patient-derived xenografts (PDXs) and genetically engineered mouse models (GEMMs).[36]

This review provides an overview of the range of *in vitro* and *in vivo* models of pancreatic cancer that are being used in preclinical research. It considers an overview of platforms for cancer research with a focus on those devoted to resistance mechanisms in PDAC and the therapeutic intervention gemcitabine. The goals of the paper are to examine the impact of each tumor resistance model for PDAC.

2. Background

Therapeutic resistance mechanisms can be tumor cell-intrinsic (present before treatment)[1], acquired during treatment by various therapy-induced adaptive responses,[2] or mediated by the tumor microenvironment (TME).[3,4,5,6] Tumor molecular and genetic heterogeneity is the main reason for the failure of conventional cancer therapy as resistance can arise from the positive selection of a drug-resistant tumor subpopulation.[5]

The high adaptability of tumors through activation of pro-survival signaling pathways and the inactivation of downstream death signaling pathways can lead to drug resistance.[2] The activation of epidermal growth factor receptor (EGFR) also serves as a resistance mechanism against chemotherapies, including treatments for pancreatic cancer, such as 5-fluorouracil, irinotecan, and nanoparticle albumin-bound paclitaxel (nab-paclitaxel).[5]

Mechanisms of resistance to cytotoxic and targeted chemotherapeutics include an increased rate of drug efflux, alterations in the drug target, activation of pro-survival compensatory signaling pathways, and ineffective induction of cell death.[5] Cell plasticity also facilitates adaptive cellular reprogramming to drive acquired drug resistance.[2]

Drug-resistance mechanisms are regulated by the TME, epithelial-mesenchymal transitions (EMTs), and microRNA.[7] Excellent reviews on the contents of the TME have been published previously[4,5,10,13,17,20,21] and from these works, it is clear that the TME consists of the extracellular matrix, cancer-associated fibroblasts, immune and inflammatory cells, tumor-associated macrophages (TAM), and blood vessels that provide refuge for cancer cells from cytotoxic agents. Additionally, epithelial cells can undergo a transition to become mesenchymal cells by losing their polarized organization and tight cell-cell junctions to change cell shape and develop a fibroblast-like morphology.[5] Studies have found a correlation between chemotherapeutic resistance and the EMT.[23,24] Similarly, microRNAs (miRNAs), a class of small non-coding RNAs that negatively regulate genes at the post-transcriptional level, have also been shown to affect drug resistance.[7] Recent studies have found a correlation between miRNA expression and resistance towards chemotherapeutic targets.[7]

Cancer research typically involves these drug-resistance mechanisms and relies on model systems, which reflect the biology of human tumors to a certain extent.[25] Models throughout cancer research history have addressed all stages of drug discovery, including target identification, toxicity, and individual patient prediction. Initial molecular biology models have increased understanding of tumor cell biology, while new model systems simulate functional processes related to the development and growth of cancer. Cell cultures, namely those derived from a cervical cancer patient Henrietta Lacks (HeLa), became the first laboratory model for cancer research in understanding tumor biology, drug identification, and drug development.[25] In addition to 2D cell cultures, other models currently in use include *in vitro* conditionally reprogrammed cell (CRC) lines, 3D cell cultures, organoids, spheroids, and tumor-on-the-chip models, *in vivo* zebrafish models, patient-derived xenograft (PDX) mouse models, genetically engineered mouse models (GEMMs), and transgenic mouse models, as well as computational or mathematical models. Considering how mechanisms for tumor hypoxia, senescence, and cytoskeletal organization vary, a current challenge in cancer research is selecting the model that best reflects the given tumor entity. Thus, the roles of cellular senescence, dormancy in tumor formation, and therapy resistance have become increasingly important and relevant in cancer research.[26] This paper will survey these topics and investigate their utility in various applications.

3. Models in Cancer Research

This section describes each of four categories of cancer research model systems: in vitro models (2D and 3D cell cultures), in vivo models (PDX, GEMMS, transgenic), proteomic models, and computational or mathematical models (Figure 1).

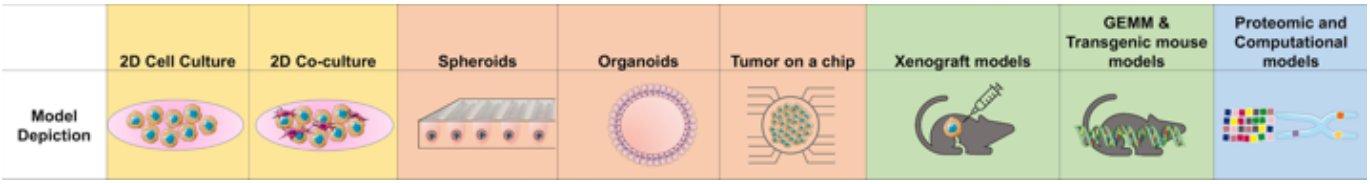

Figure 1. Models in Cancer Research

3.1 In Vitro Models

3.1.1 2D Cultures

The use of *in vitro* models has allowed for preclinical and translational studies of tumor mutations, aberrations, and responses to therapeutic agents.[27] 2D cell culture models are inexpensive and easy to generate and maintain, making them the mainstay for cancer research. Still, these models have limitations, including induced alterations in cell morphology that translate to changes in gene and protein expression. 3D cell cultures have partially overcome this shortcoming.[27]

Conditionally reprogrammed cells (CRCs) overcome senescence to produce immortalized primary cell cultures through a method described by Liu et al. (the Georgetown method), which combines irradiated mouse fibroblasts as a feeder cell layer with the use of a Rho kinase (ROCK) inhibitor.[28] Cells can be derived from both normal and cancer tissues and grown indefinitely under these conditions while maintaining a normal phenotype.[25] The irradiated mouse fibroblasts maintain telomerase reverse transcriptase (hTERT) expression to prevent chromosome shortening and tumorigenic cellular transformation of normal cells. Furthermore, the use of ROCK inhibitors maintains the undifferentiated and proliferative state of epithelial cells. In combination with the feeder cells, ROCK inhibitors prevent the transformation or senescence of cultured cells.[25] Thus, Liu et al. demonstrated that irradiated murine fibroblasts and a ROCK inhibitor are essential for both initial survival, unlimited expansion, and senescence prevention.[28]

Yuan et al. were one of the first to use CRCs to generate cell cultures from the patient's normal and tumorous lung tissue and study tumor progression in recurrent respiratory papillomatosis.[29] By co-culturing the primary cells with J2 murine fibroblast cells and a medium containing ROCK inhibitor Y-27632, they were able to detect a mutation of HPV-11 in the CRCs that may contribute to aggressive clinical behavior. Furthermore, the CRCs were used in screening potential drug therapies, which allowed for the identification of vorinostat as a potential therapy for patients with recurrent respiratory papillomatosis. Thus, this method of generating cell cultures from many epithelia can be used in personalized medicine and to study other cancers and diseases. CRCs have also been used in regenerative medicine, drug sensitivity testing, gene expression profiling, and xenograft studies.[29,30,31,32]

Recent models with CRCs include those for prostate cancer, which aimed to define potential new therapies and observe drug sensitivity and resistance. Naeem et al. modeled prostate cancer using CRCs of normal and prostate cancer (PCa) cells derived from treatment-naive patients with primary PCa.[31] Using an integration of an in silico proteochemometric network pharmacology platform and *in vitro* methods with CRCs, the researchers examined drug response in sensitivity assays on PCa CRCs and predicted novel applications for PCa chemotherapies, including broad applicability to rapidly identity and test approved drugs. Additionally, Tricoli et al. presented a 3D non-spheroid model using CRCs for normal and tumor-derived PCa CRCs to describe the combined effects of a multi-dimensional transwell platform and define culture media on PCa cellular proliferation, differentiation, and signaling.[33] The use of a transwell-dish culture method (TDCM) enables multi-dimensional culturing of PCa CRCs, allowing for a more mature, stratified prostate epithelial phenotype that can be used for basic and translational studies of PCa.[34]

3.1.2 3D Cultures

While 2D *in vitro* cell culture models are widely used for studying the basic biology and tumorigenesis of various cancers, 3D models more accurately mimic the native cancer tissue by preserving cellular heterogeneity and replicating some of the specific biochemical and morphological features of the corresponding tissue

in vivo. This similarity to *in vivo* tissue provides an advantage in the model for examining morphology, tumor microenvironment, invasion, metabolism, and cell-environment crosstalk that influences gene expression and cell behavior. Furthermore, 3D cell cultures serve as a model for experimental therapy studies using radiotherapy, chemotherapy, and cell- and antibody-based immunotherapy.[26] Several reports demonstrate that cancer cells can grow in non-adherent conditions, forming 3D structures, or spheroids, that show increased resistance to drugs frequently used for cancer treatment.[27] Due to their flexibility in mimicking tumor microenvironments through modifying cell culture conditions, 3D culture models are useful tools for studying cancer development and potential targets for therapeutic intervention.

The most innovative and promising approach for *in vitro* modeling employs tumor tissue **organoids**, which are self-organizing, multicellular structures derived from primary tissue and grown in well-defined conditions. Several methods for developing organoid lines and biobanks have been used for prostate cancer,[35] pancreatic cancer,[36] and colorectal cancer[37] for in vitro drug testing. While drug sensitivity is usually exploited using rapid *in vitro* screening through a short-term culture of tumor sections and *in vivo* screening through xenotransplantation of the tumor into immunodeficient mice, organoid technology bridges these two approaches to produce a feasible medium-throughput drug screen on patient-derived organoids (PDOs).[35,37] These 3D organoids can maintain their complex architecture and reproduce their marker expression, allowing several research groups to study tumor development and test for drug efficacy.[6,27,36,37,38,39,40] Organoid cultures typically use Matrigel or collagen and support the growth of both normal and cancer tissue such that cultures can be manipulated at the genetic level by transfection.[27]

When cultured with stroma and fibroblasts, organoids have the potential to simulate the full spectrum of patient cancer progression and study normal cells, preinvasive carcinomas, and metastatic cells.[6,41,42] Furthermore, PDO co-cultures with immune cells,[43,44] cancer-associated fibroblasts,[45] and stellate cells[42] can identify tumor microenvironment characteristics and determine mechanisms for resistance to traditional and investigational drugs.[6]

Organoids are generated rapidly and reliably, especially with endoscopic ultrasound (EUS)-guided tissue acquisition at the time of initial diagnosis,[41] making them more usable for patients who need targeted treatment as quickly as possible. The development of PDO biobanks also greatly expands the type of patient samples that can be propagated and studied, and these samples can then be used to accurately predict drug responses in a personalized treatment setting.[6]

Tumor- or cancer-on-a-chip (CoC) models have been used more recently to dissect the role of tumor microenvironment cues and their role in metastasis.[22] These microfluidic chip device models enable control over local gradients, fluid flow,[46] tissue mechanisms[111], and composition of the local environment through micrometer- to millimeter-sized compartments and microchannels.[22] The small chamber for cell culture creates a niche in which tumors can grow, develop, and interact in a specified microenvironment.[22]

By making it possible to manipulate the variables listed above, CoC models overcome some of the limitations of 2D or 3D cell cultures and animal models. The CoC model used depends upon the particular tumor microenvironment cues that researchers aim to understand. The many different types of CoC models include 2D chips, lumen chips for CAFs,[47] compartmentalized chips for TAMs,[48,49,50] CAFs,[32,51] Y chips for CAFs,[52] and membrane chips. Many models have a gel-fluid interface lined with endothelial cells.[48,53] CoC models have helped better understand the invasion-related interactions between CAFs and cancer cells, as well as how activation of TAMs enhances cancer invasion through live observation of microenvironmental dynamics.

3.2 In Vivo Models

An *in vivo* model, **zebrafish**, serves as an intermediate of cell culture models and mammalian models. This zebrafish model allows for extracorporeal fertilization and has a well-researched translucent embryo, short generation time, as well as a well-developed genetical toolbox, which makes the zebrafish ideal for studying development.[26] Zebrafish melanoma models have shown that the cause of cancer formation is the de-differentiation of epithelial cells to form embryonic neural crest cells.[26] Moreover, zebrafish xenograft models can also be used to determine the tumor-forming capacity of PDAC CRCs and assess whether chemotherapeutic resistance was retained *in vivo*.[54]

Therapy-resistance ***in vivo*** **animal models** mainly use mice due to optimal short generation time (10 weeks), average life expectancy (2.5 years), the possibilities for reverse genetics, and the frequent occurrence of cancer in the absence of oncogenic agents. The use of mouse models dates to the late 1960s and the development of immune-deficient mouse strains.[26]

Patient-derived xenotransplants (PDX) use chemotherapy-naive tissue obtained from surgery or biopsies and transplant them into immune-deficient mouse strains, like nude or severe combined immunodeficiency (SCID). [26,55,56,57,58,59,6] Immunocompetent and immunodeficient mice with xenografted tumors are traditionally transplanted subcutaneously or orthotopically.[61,62] These models are widely used due to their availability, low cost, and ability to mimic attributes of human malignancies by recapitulating neoplastic cell architecture and conserving genetic and phenotypic biology at the histological and molecular models.[55] This makes PDX models the favored method of identifying drugs that significantly inhibit tumor growth, validating tumor biomarkers, and predicting treatment outcomes.[55]

Highly sophisticated **transgenic mouse models** allow researchers to look at the early stages of tumor development and constitutively or conditionally induce the expression of an oncogenic mutation at a specific time and in a specific organ using conventional methods, such as retroviral infection, microinjection of DNA constructs, and the "gene-targeted transgene" approach.[26,63,64] The use of transgenic models has been important in studies evaluating the development of resistance to therapy.[63] Knockout transgenic mice, in which the gene is depleted or silenced to cause a loss of gene function, are a powerful tool for assessing the potential validity of a targeted therapy because the targets can be precisely inactivated in the developing or developed tumor.[63] Recently, the use of CRISPR/Cas9-based transgenic models has allowed for more effective systems to study human cancers.[63] Due to advances in immunotherapy that have illuminated the importance of immune response in tumor progression and treatment, new PDX models, namely those that interact with the human immune system, are necessary.

Humanized mouse xenograft models replace the mouse immune system with a human immune system. These models transplant $CD34^+$ human hematopoietic stem cells (HSCs) into mice to produce human blood cells. They are potentially valuable models for new immunotherapies because they mimic tumor heterogeneity, the tumor microenvironment, and crosstalk between the tumor and stromal/immune cells.[63]

Wang et al. developed human hematopoietic and immune systems in mice transplanted with human (h)CD34+ hematopoietic progenitor and stem cells.[65] After implanting the PDX of non-small cell lung cancer (NSCLC), sarcoma, bladder, cancer, and triple-negative breast cancer into the humanized NSG (huNSG) mice, the researchers discovered that tumor growth curves in the humanized mice were similar to those in non-human immune cell-engrafted NSG mice. Additionally, treatment with pembrolizumab, which targets programmed cell death protein 1, produced significant growth inhibition in PDX tumors in huNSG mice but not in NSG mice. These results suggest that tumor-bearing huNSG mice can serve as a novel platform for testing the efficacy of immunotherapies. Future data collected

from these humanized mouse models will enable the development of predictive cancer biomarkers of response to chemotherapies. Chang et al. described a novel orthotopic renal cell carcinoma (RCC) xenograft humanized mouse model as an improved model to evaluate *in vivo* anti-tumor capabilities of fully human monoclonal antibodies for RCC therapy.[66,55]

Genetically engineered mouse models (GEMM), which are produced by modifying specific genes associated with cancer, provide an authentic, bridging model to patients, as the tumors created are aggressive, heterogeneous, and stromal (desmoplastic) in nature.[55] In addition to sharing similar genetic, phenotypic, and physiological characteristics with humans, GEMMs also suffer from typical cancer symptoms (bodyweight loss, cachexia, etc.) and the spontaneous formation of distant metastasis.[55] The development of these symptoms makes it possible to simulate different stages of tumorigenesis.

3.3 Computational Models

Therapy resistance has also been studied through **computational, theoretical, and mathematical models.** The possibility of using bioinformatics models to create personalized medicine applies datasets to tumor material, the genome of the cancer patient, and metabolic pathways. Mathematical models based on partial differential equations often deal with the growth of cancer cell lines *in vitro* while considering parameters like initial cell density and concentrations of cell cycle inhibitors.[26] These simulations can predict the conditions in which the tumor cells will die out.

3.4 Gemcitabine Resistance and PDAC models

GEM is a nucleoside analog used in chemotherapy for non-small cell lung, pancreatic, bladder, and breast cancers.[72] Since its approval by the FDA in 1996, GEM has been used as a first-line treatment for patients with locally advanced (nonresectable Stage I/II) or metastatic (Stage IV) PDAC and remains the first-choice treatment for PDAC. GEM inhibits DNA synthesis, acting as an analog of cytidine to prevent chain elongation, and further induces apoptosis in cancer cells via caspase signaling.[73] However, GEM treatment resistance along with the poor pharmacokinetic profile of GEM (8-12 mins in humans due to rapid metabolism) has resulted in poor treatment outcomes and drug resistance development over time.[71,74]

4. Gemcitabine Resistance and *In Vitro* Models

4.1 Gemcitabine Resistance and 2D Cultures

Panc-1, MiaPaCa-2, SW1990, and Capan-2 are 2D cell cultures that remain platforms used to study GEM resistance mechanisms and improve the efficacy of GEM in combination with other therapies.[55,72] These cancer cells have the capability to generate high-fidelity *in vitro* models to explore the efficacy of anticancer drugs. Pancreatic cell lines (Capan-1, T3M4, MiaPaCa-2) with acquired GEM resistance (GEM-R) have elucidated resistance mechanisms that include signaling crosstalk to increases glucose uptake[75] and kinase inhibitors capable of inhibiting the growth of GEM-resistant MiaPaCa-2 cells.[76]

Affram et al. investigated the cytotoxic effects of an alternative drug delivery system in the form of GEM-loaded solid lipid nanoparticle (GEM-SLN) on patient-derived primary pancreatic cell lines (PPCL-46) and MiaPaCa-2 pancreatic cancer cells.[72] Solid lipid nanoparticles (SLNs) are nanocarriers that can be used as an alternative drug delivery system to improve therapeutic effectiveness for drugs, like GEM, with a short half-life that requires continuous parenteral administration. The researchers' cytotoxicity studies found a greater cytotoxic effect of GEM-SLN treated PPCL-46 than of GEM hydrochloride (GEM-HCl) treated PPCL-46 cultures. A similar trend of higher GEM-SLN inhibition was found in MiaPaCa-2 cultures as well. These results indicate the potential for enhanced GEM delivery and improved anticancer activity through GEM-SLN. Additionally, GEM-SLN is effective on both PPCL-46 cultures and established commercially available cell lines,

indicating moderate effectiveness in addressing the heterogeneity of pancreatic cancer cells.

4.1.1 Gemcitabine Resistance and 2D Co-Cultures

Pancreatic stellate cells (PSC) are often used in co-culture experiments with PDAC cells to illuminate microenvironmental issues and provide a more accurate model than a single epithelial monolayer model.[77,78,79,80] PDAC often displays a dense desmoplastic stroma, which has been associated with chemoresistance and inhibition of drug penetration.[81] 3D matrices of PSC cells stimulate life-like settings and studies on these matrices reveal that additional tumor stroma components (fibroblasts, macrophages, immune cells, and endothelial cells) play an important role in therapy resistance.[55]

Karnevi et al. highlighted the role of PSCs in the epithelial-mesenchymal transition (EMT) as an intrinsic part of cancer progression that downregulates epithelial phenotype and cell-cell adhesions.[24] Co-cultures of immortalized primary PSCs with Panc-1, MiaPaCa-2, and BxPC-3 revealed down-regulated E-cadherin levels and increased expression of vimentin, both of which indicate the role of PSCs in modulating the epithelial-mesenchymal transition (EMT).

More recently, Xiao et al. found increased expression of Yes-associated protein 1 (YAP1), a protein known to induce cancer-associated fibroblast activation in liver and breast tissues, in PSCs.[82] A co-culture with MiaPaCa-2 and human PSCs from residual surgical specimens revealed that YAP1 may play a critical role in the regulation of PSC activation, indicating a novel rationale for targeting YAP1 to reprogram the PDAC microenvironment.[82] In addition to YAP1, it is speculated that secreted protein acidic and cysteine-rich (SPARC), a matricellular glycoprotein used in ECM assembly and cell-matrix communication during tumor progression, may also be related to PSC activation. High levels of SPARC expression in stromal cells indicated poor prognosis of PDAC patients and acted as a negative predictive biomarker in patients treated with GEM based chemotherapy.[81]

New cell lines, of human and murine origin, are continually being established and characterized for use in screening novel drug candidates and elucidating signaling pathways or (epi)genetic events involved in tumor development, progression, and the outcomes of therapy.[55]

4.1.2 Gemcitabine Resistance and Conditionally Reprogrammed Cell Lines

Studies on PDAC CRCs have been investigated with nab-paclitaxel, but not GEM.[34] Drug sensitivity screens were conducted for cultures of muscle-invasive bladder cancer that revealed sensitivity to GEM. These results showed that CRCs are a feasible platform for personalized drug sensitivity testing for bladder cancer.[83]

4.2 Gemcitabine Resistance and 3D models

The application of 3D models has been a growing trend in PDAC studies, especially with developing models for drug screening. Oftentimes, chemotherapeutics that were effective in 2D models do not remain effective in 3D models. Different IC_{50} values between 2D and 3D models point to the clear discrepancy between the commonly used 2D drug screening versus the more complex 3D and co-culture models. Furthermore, 2D cell culture is known to not fully recapitulate tumor biology.[73] 3D culture models better reflect actual tumor drug responses and aid in the identification of novel compounds that are more effective.[76] Differences between pancreatic cell lines in the morphology of both 2D and 3D cultures can be attributed to differences in origination site. BxPC-3 obtained from PDAC lacks metastatic potential, while MiaPaCa-2 and PANC-1, both of which are also derived from PDAC, demonstrate a predisposition to metastasis.[84] COLO-357 and AsPC are obtained from metastatic sites, while T3M4 cells are derived from the lymph node metastatic mass and resemble BxPC-3.[84]

Many models are based on co-cultures with other cell types or cells believed to contribute to the transformed phenotype and invasiveness of the

cells. Notably, stromal-tumor cell interactions are actively studied in PDAC drug resistance.[72] Recent research considers the tumor microenvironment that has been associated with metastatic progression and vascularization. These complex systems are easier to model using 3D structures, such as multicellular tumor spheroids (MCTS) [84] and organoids.[6] While spheroids and organoids both form 3D models, they differ in their morphology. Derived from tissue or cancer stem cells, spheroids grow in a minimum serum-free medium. They trigger an oxygen and nutrient gradient that leads to massive cell death in the center of the structure, which is why they cannot form tissue-like structures. In contrast, organoids require stem cell niche factors and extracellular matrices, which allow the organoids to differentiate and self-organize. Additionally, organoids do not exhibit a hypoxic or nutrient gradient.[85]

4.2.1 Gemcitabine Resistance and Tumor Spheroids

MCTS serve as models of PDAC tumors superior to flat cell monolayers (2D cultures) due to different geometry that leads to changes in nutrient and oxygen turnover as well as cell crosstalk. Current MCTS models are derived from several epithelial cell lines, including AsPC-1, BxPC-3, Capan-1, MiaPaCa-2, and PANC-1.[84] Svirshchevskaya et al. identified three types of MCTS: while Type I, BxPC-3, and T3M4 formed a small number of large and dense spheroids, Type II, COLO-357, and AsPC-1, generated by E-cadherin contacts, formed multiple and loose MCTS of different sizes[84]. Type III, MiaPaCa-2, and PANC-1 cells grew as floating monolayer films as they were unable to form MCTS. Cell growth for these 3D cultures and monolayers revealed a dramatic (2 order) reduction in cell proliferation for 3D type I cell line cultures treated with GEM. Drug resistance in the type I 3D cultures was found to be associated with a quiescent state (decreased proliferation) and a high level of spontaneous apoptosis in cells. Meanwhile, type II and III MCTS had comparable sensitivity to the antitumor drugs.

Ware et al. describe the generation of a 3D PDAC *in vitro* micro-tumor model that encompasses a stromal component using PSCs, which are myofibroblast-like cells located in the exocrine areas of the pancreas.[62] PSCs play a role in normal pancreatic architecture as they secrete extracellular matrix (ECM) components and are the principal source of fibrosis in the stroma. Additionally, sequestration of chemotherapeutic agents, such as GEM, occurs in the tumor stroma, effectively reducing the amount of drug that can reach cancer cells. Their PDAC stroma spheroids model presented decreased cytostaticity of GEM when compared with spheroids grown without PSCs. A study by Lee et al. corroborates these findings.[86] This model will allow for improved knowledge of PDAC biology and can be used to investigate pathways that can be therapeutically targeted to inhibit PSC activation and subsequent development of fibrosis in PDAC.

4.2.2 Gemcitabine Resistance and Organoids

Pancreatic ductal organoids are ex-vivo models that can be established using very small biopsies, such as fine-needle aspirates [36,41] and allow for the study of localized, advanced, and metastatic patients. Boj et al. established organoid models from normal and malignant murine and human pancreas tissues to investigate the pathogenesis and address the deficiency in a comprehensive 3D cell culture model of murine and human PDAC progression.[36] This model was used to identify genetic drivers, therapeutic targets, diagnostics, and progression for PDAC.

Tiriac et al. used EUS fine-needle biopsy (EUS-FNB) sampling to rapidly establish human PDAC organoids within 2 weeks of the EUS procedure and assessed the feasibility of this model in creating personalized treatment strategies at the time of initial tumor diagnosis and over the course of a patient's treatment. PDAC patients are ideal for EUS-FNB derived organoids as most patients will not undergo surgery, all patients need a tissue diagnosis before therapies are initiated, and only a small amount of tissue is needed for organoid creation. Successful organoid generation is

necessary for developing personalized medicine platforms for PDAC patients.[41]

These 3D primary ex vivo culture systems model a spectrum of tumor stages and have elucidated important disease progression findings. Profiling with next-generation sequencing of DNA and RNA in combination with pharmacotyping can be used to predict responses in PDAC patients and provide a pathway for prioritizing therapy.[87]

4.2.3 Gemcitabine Resistance and Tumor-on-a-chip

To overcome limitations of traditional disease model systems, organ- or tumor-on-a-chip systems aim to fully recapitulate the physiology and microenvironment of tissues through spatial and fluid control of tissue architecture.

Kramer et al. examined the effect of interstitial flow on GEM resistance in PDAC using a 3D microfluidic platform. Interstitial pressure and flow are hallmarks of PDAC pathogenesis.[73] The study used 3D cultures of S2-028 cells, a non-metastatic pancreatic cancer line, and found an increase in mRNA expression of 5 multidrug resistance proteins (MRPs) when the PDAC cells were subjected to interstitial flow. This flow-induced MRP expression hints towards another factor that contributes to GEM resistance via elevation of drug efflux transport.

5. Gemcitabine Resistance and *In Vivo* Models

5.1 Gemcitabine Resistance and Xenograft Models

Cell line-derived xenograft models, such as BxPC-3, MiaPaCa-2, and Panc-1 xenografts, are popular for drug screening and resistance studies in PDAC that can be applied to increasing patient survival. Novel tumor growth-inhibiting compounds derived from gemcitabine have been applied to Panc-1 and MiaPaCa-2 bearing mice.[88]

Xenograft tumor assays have demonstrated that the tetracyclic diterpenoid compound Ordonin overcomes gemcitabine resistance in gemcitabine-resistant Panc-1 cells (PANC-1/GEM) through suppressing tumorigenicity in nude mice. Additionally, a combination treatment of oridonin and gemcitabine decreased tumor growth.[88] Gemcitabine resistance is mediated by a special AT-rich sequence binding protein 1 (SATB-1), a chromatin organizer that is secreted by cancer-associated fibroblasts (CAFs).[89] SATB-1 plays a vital role in the proliferation capacity of SW1990 tumor cells in mouse xenograft models. SATB-1 has been associated with poor prognosis and tumorigenesis in pancreatic cancer.[89,90,91]

Xenograft models have also revealed the role of Prolactin receptors in suppressing tumor growth. Dandawate et al. studied the role of prolactin receptors in PDAC through the injection of a diphenylbutylpiperidine-class antipsychotic drug, penfluridol, which binds to prolactin receptors.[92] Penfluridol slowed the growth and volume of tumors in Panc-1 xenografts in athymic nude mice and PDX in immunodeficient NSG. Western blot analyses suggested that penfluridol induces autophagy-related proteins p62, ATG-5, ATG-7, ATG-12, LC3B, and beclin-1 to suppress PDAC tumor growth.[92] Additionally, cysteine transport, xCT, is key to tumor growth. Tumor xenograft growth of genomic knockouts of xCT was delayed but not suppressed, indicating the key role of xCT and the presence of additional mechanisms for cysteine homeostasis *in vivo*.[93]

Patient-derived xenograft (PDX) models are widely used for various solid tumors. Typically, chemotherapy-naive tumor tissue obtained from surgery or biopsies is transplanted directly into immuno-deficient mice.[55] PDX models for pancreatic cancer are used to identify drugs that significantly inhibit tumor growth or to validate prognostic biomarkers, as these models are highly representative of their respective tumors due to a high degree of genetic stability observed by short tandem repeat (STR) profiling and mutation analysis.[94] Well-defined PDX collections can be used to associate biomarkers with drug sensitivity and resistance to facilitate precision cancer medicine. Most publications on pancreatic PDX models describe the establishment, characterization, and preclinical application of

PDXs, but they have yet to be applied towards clinical studies.

Tang et al. used GEM treated PDX models to show that m^{6A} demethylase ALKBH5 is downregulated. Overexpression of this demethylase sensitizes PDAC cells to chemotherapy. Thus, lower levels of ALKBH5 predict poor clinical outcomes in PDAC and other cancers.[95] Wei et al. used *in vivo* CRISPR gene knockout screening in PDX mice to identify effective lethal drug combinations that synergize with GEM for treating PDAC.[96] Using a clinically relevant PDX model of PDAC with a patient tumor being propagated within the pancreas of athymic nude mice, they screened for chromatin regulators whose depletion may create conditional lethality with GEM. They found that inhibition of the protein PRMT5 led to synergistic vulnerability of PDAC cells to GEM. *PRMT5* has been considered as a critical driver of cancer progression for multiple advanced-stage cancers. This study suggests that GEM treatment combined with inhibition of PRMT5 will have stronger effects and selectivity towards PDAC.[96]

5.2 Gemcitabine Resistance and Genetically Engineered/Transgenic Models

A major drawback of PDX models is the use of immunodeficient mice that lack a competent immune system to investigate immunotherapeutics. Tumor cells used in PDX have also been passaged extensively *in vitro*, limiting tumor cell heterogeneity and potential biological relevance. Thus, genetically engineered mice that spontaneously develop PDAC are appealing for drug discovery, especially since tumors arise in competent and fully intact immune systems.

GEMMs are similar to humans in terms of genetic, phenotypic, and physiological characteristics. Models include KC ($Kras^{LSL.G12D/+}$ and PdxCre) mice and KPC mice that have been used to investigate the influence of Kras.[55] KC mice have normal pancreatic organogenesis and develop intraepithelial neoplasia (PanIN) that eventually progress to PDAC. KPC ($Kras^{LSL.G12D/+}$, $p53^{R172H/+}$, and PdxCre) mice have a conditional expression of the R72H mutation in the p53 gene in the $Kras^{G12D}$ context. KPC is the most extensively studied genetic model of PDAC for the evaluation of immunotherapy.[97] At least 40 GEMM have been generated for analysis of gene function in PDAC.[98,99]

Tadros et al. observed an increase in fatty acid synthase expression that corresponded with increased disease progression in PDAC GEMMs.[100] Based on analysis and identification of the lipid metabolism pathway to be the most significantly enriched in tumors from patients with PDAC, they manipulated the fatty acid biosynthesis pathway. Fatty acid synthesis is also regulated by multiple transcriptional regulators, including c-MYC, which is significantly amplified in PDAC.[54] Through treatment with orlistat, the researchers demonstrated a way to overcome GEM resistance in pancreatic cancer by regulating endoplasmic reticulum stress and stemness.

Buccholz et al. showed that depletion of pharmacological tumor-associated macrophages (TAMs) improves therapeutic response to GEM in KPC mice.[101] Macrophages are abundant in fibro-inflammatory TME of KC and KPC mice, which has been shown to correlate with a worse prognosis in PDAC.[102] Following enrollment of KPC mice that had developed pancreatic tumors with liposomal clodronate to deplete intratumoral TAMS and GEM, they demonstrated improved efficacy of GEM in the KPC. These results point to another instance of the role of the TME in GEM resistance.

Principe et al. evaluated the effects of prolonged GEM treatment using KPC mice and found increased CCL/CXCL cytokine/chemokine secretion and upregulation of immune surface proteins, including transforming growth factor β (TGFβ)-associated signals, in the tumor stroma.[103] TGFβ-associated signals confer drug-resistant phenotypes to neighboring stromal cells and further enhance the production of inflammatory cytokines/chemokines.

Halbrook et al. examined how tumor-associated macrophages (TAMs) drive resistance

to GEM in PDAC cell lines. They found that TAMs release a spectrum of pyrimidine species, such as deoxycytidine, that inhibit GEM through direct competition, hindering drug efficacy. KPC mice treated with GEM combination treatments had prolonged survival compared to control mice, indicating that inhibiting macrophage recruitment has the potential to improve current PDAC therapies, as seen with FOLFIRINOX.[104]

Özdemir et al. used transgenic (*Ptf1acre/+;LSL-KrasG12D/+;Tgfbr2flox/flox*) mice with deleted αSMA+ myofibroblasts in pancreatic cancer. Myofibroblast depleted tumors did not respond to GEM and resulted in multiple adverse outcomes. Their results suggest that fibrosis associated with myofibroblasts and type I collagen constitutes a protective response from the host rather than offering an oncogenic supportive role.[105]

6. Gemcitabine Resistance and Proteomic and Computational Models

GEM resistance has also been investigated using proteomic and computational models. While biological processes of drug resistance have been described before, proteomics serves as a powerful tool for better understanding molecular mechanisms of GEM resistance.[106] Proteomics investigates proteins whose expressions differ between drug-sensitive and drug-resistance cells. This method can provide system-wide views of signaling networks to better understand drug mechanisms of actions and interactions.[106] Proteomics also provides the knowledge needed to identify biomarkers and for targeting specific protein pathways.[106]

Chen et al. examined mechanisms associated with GEM-induced resistance using 2D-DIGE and MALDI-TOF mass spectrometry and compared the proteomic alternations of a panel of differential GEM-resistant PANC-1 cells and GEM-sensitive pancreatic cells. They found that 33 proteins were differentially expressed between GEM-sensitive and GEM-resistant cells.[107]

Zhu et al. studied GEM with birinapant in PDAC. They identified 4069 drug-responsive proteins and quantified them in a time-series proteome analysis to highlight and quantify signaling pathways. Pathways related to DNA damage response, DNA repair, anti-apoptosis, pro-migration/invasion were implicated as underlying mechanisms for gemcitabine resistance. This study identified promising drug targets for future investigation.[106]

Law et al. analyzed clinical PDAC liver metastases with quantitative proteomics. Their proteomic analysis of molecular signatures unique to the disease subtypes identified GEM-induced alterations in proteins, such as serine hydroxymethyltransferase 1, that are associated with drug resistance.[108] PDAC subtypes can be characterized using proteomics and can be used to inform first-line cancer treatment. These efforts illustrate the potential of applying proteomics to improve PDAC subtype classification and therefore early detection and treatment of PDAC.

Computational modeling of gemcitabine-based therapies has also been conducted to determine optimal intervention strategies.[109] Furthermore, transcriptomics has been implemented to better under the tumor microenvironment and chart changes in the fibroblastic landscape in PDAC progression.[110]

7. Conclusion

Models are essential to addressing issues of drug resistance in cancer phenotypes. While GEM resistance in PDAC continues to result in dire outcomes for patients, models can serve as a step towards elucidating resistance mechanisms to improve treatment protocols. The number of models currently in use can reproduce a wide range of tumor mechanisms to ultimately understand factors such as cell-cell interactions and the tumor microenvironment. The use of various *in vivo*, *in vitro*, proteomic, and computational models will be crucial in making a clinical impact and

benefiting PDAC patients with better platforms for treatment and diagnosis.

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About the Authors

Hyunwook Nam

Hyunwook Nam is a junior majoring in neurobiology. He is interested in psychological disorders and neurodegenerative diseases. Previously, Nam has worked in Dr. Bill Rebeck's lab and has interned at the Korea Brain Research Institute.

Vidya Mullangi

Vidya Mullangi is a junior from Burlington, Iowa studying neurobiology in the College. She explores neurodegeneration in Dr. Italo Mocchetti's lab and enjoys providing music therapy for cancer patients. Mullangi plans to attend medical school after she graduates.

Lauren Cox

Lauren Cox is a junior majoring in Neurobiology in the College. Lauren currently works in the Mocchetti Lab of Neuroscience. After graduating, Cox hopes to apply to medical school while maintaining her involvement in neurobiology-based research.

Alexa Asch

Alexa Asch is a junior in the College at Georgetown University. She is majoring in neurobiology and minoring in disability studies, and she is on the pre-med track. In the future, she hopes to continue doing research and being involved in medicine.

Nikita Shah

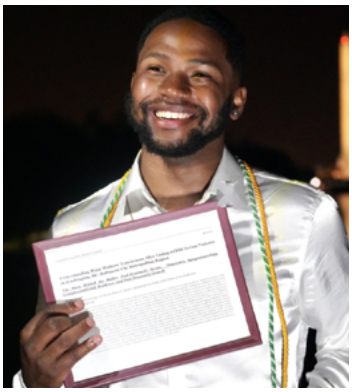
Nikita Shah is a junior at Georgetown University majoring in Neurobiology and minoring in Spanish. She is on the pre-med track and plans to pursue a career in pediatric medicine.

Clarisa Mendoza

Clarisa Mendoza, born and raised in Caracas, Venezuela, is a junior in the College majoring in neurobiology and minoring in disability studies. In the future, she would love to attend medical school and expand her knowledge of neuroscience.

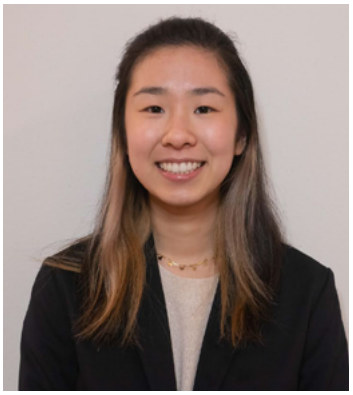
About the Authors

Denzell Brown



Denzell Brown is a Washington D.C. native and a senior at Georgetown University. He is majoring in psychology and is devoted to the lived experiences and psychological healing of Black women in the DC-metropolitan area.

Britney He



Britney He is a junior in the College majoring in biology and minoring in public health. He currently researches cancer models in Dr. Chris Albanese's lab. She is on the pre-med track and hopes to pursue a career in medicine.

Sofia Flynn

Sofia Flynn is a mathematics major from Virginia and a junior at Georgetown University. Her academic interests lie in number theory and cryptography. Sofia plans to pursue graduate studies in mathematics and has ambitions of contributing to the burgeoning world of post-quantum cryptography.

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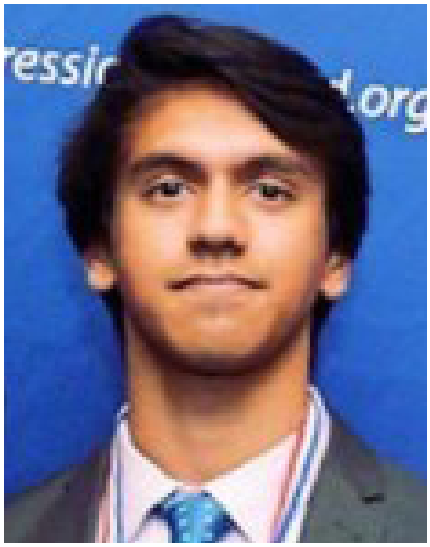

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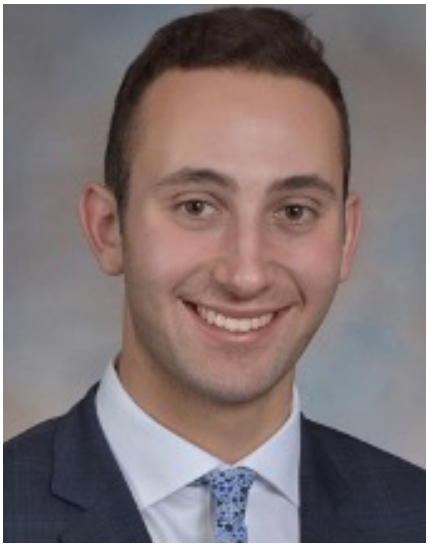

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