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Evaluation of XRCC2 gene`s methylation pattern in Breast Cancer

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Abstract

Breast cancer (BC) is a leading cause of morbidity and mortality among women, with its development influenced by genetic factors such as mutations in the XRCC2 gene, a key player in DNA repair via homologous recombination. This study aimed to analyze XRCC2 methylation rate in promotor region in BC tissues as epigenetic factor and compared to normal breast tissues to elucidate its potential role in BC pathogenesis. An observational analytical study with a case-control design was conducted at Zheen International Hospital, Erbil, Iraq, from 2021 to 2024. The study included 44 adult women diagnosed with BC. The X-ray repair cross-complementing group 2 (XRCC2) gene encodes a member of the RecA/Rad51-related protein family that participates in homologous recombination to maintain chromosome stability and repair DNA damage. This gene is involved in the repair of DNA double-strand breaks by homologous recombination and it functionally complements Chinese hamster irs1, a repair-deficient mutant that exhibits hypersensitivity to several different DNA-damaging agents. In this study methylation status was determined using methylation-sensitive restriction enzyme digestion PCR. The XRCC2 promoter region underwent DNA methylation analysis via Methylation-sensitive restriction enzyme digestion PCR (MSRE-PCR). This involved digesting genomic DNA with a specific enzyme sensitive to methylation, followed by PCR amplification using gene-specific primers. The current study found a 7% methylation rate for the XRCC2 gene in tumor tissue, with no indication of methylation in the XRCC2 promoter region, suggesting limited regulation by methylation. further analysis is mandatory to better understand and confirm our preliminary findings.

Keywords: Breast cancer, XRCC2, Epigenetics, Methylation

Introduction:

Breast cancer (BC) is one of the most common (with 2.26 million cases in 2020) and the second leading cause of death (with 685,000 deaths in 2020) from cancer in women (Wilkinson and Gathani, 2022). It is a complex and heterogeneous disease, with various genetic and environmental factors contributing to its development and progression (Abiola et al., 2024). One of the key aspects of BC research is the study of DNA repair genes, as they play a crucial role in maintaining genome integrity and preventing the accumulation of mutations that can lead to cancer (Moon et al., 2023).

Among these genetic elements, the X-ray repair cross-complementing group 2 (XRCC2) gene has emerged as a candidate of interest due to its involvement in DNA repair mechanisms. The XRCC2 gene is integral to the homologous recombination (HR) repair pathway, a critical system for maintaining genomic stability and repairing DNA double-strand breaks, which, if left unrepaired, can lead to tumorigenesis (Andreassen and Hanenberg, 2019, Prime et al., 2024).

It appears that while a combination of mutations and copy number changes determine the type of breast cancer, epigenetic alterations may be the primary initiators of cancer development

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(BYLER, et al., 2018). Recently, there have been many studies focusing on defining these changes, and this has led to significant progress towards understanding how various epigenetic alterations, such as histone modifications, DNA methylation, and miRNA expression, ultimately affect gene expression (BYLER, et al., 2018)

Epigenetic means inheritance of phenotypic changes in a cell or organism that do not result from changes in the nucleotide sequence of DNA. Can be due to positive feedback loops of transcription regulators or to heritable modifications in chromatin such as DNA methylation or histone modifications. In vertebrate cells, the methylation of cytosine provides a mechanism through which gene expression patterns can be passed on to progeny cells. The methylated form of cytosine, 5-methyl cytosine (5-methyl C), has the same relation to cytosine that thymine has to uracil, and the modification likewise has no effect on base-pairin . DNA methylation in vertebrate DNA occurs on cytosine (C) nucleotides largely in the sequence CG, which is base-paired to exactly the same sequence (in opposite orientation) on the other strand of the DNA helix. Consequently, a simple mechanism permits the existing pattern of DNA methylation to be inherited directly by the daughter DNA strands. (Alberts et al., 2014)

DNA methylation has several uses in the vertebrate cell. A very important role is to work in conjunction with other gene expression control mechanisms to establish a particularly efficient form of gene repression. DNA methylation helps to repress transcription in several ways. The methyl groups on methylated cytosines lie in the major groove of DNA and interfere directly with the binding of proteins (transcription regulators as well as the general transcription factors) required for transcription initiation. In addition, the cell contains a repertoire of proteins that bind specifically to methylated DNA. The best characterized of these associates with histone modifying enzymes, leading to a repressive chromatin state where chromatin structure and DNA methylation act synergistically (Figure 7–45). One reflection of the importance of DNA methylation to humans is the widespread involvement of “incorrect” DNA methylation patterns in cancer progression. (Alberts et al., 2014)

Recent studies have suggested that alterations in the expression levels of XRCC2 may be associated with the development of certain cancer types, including BC (Shi et al., 2022, Liu et al., 2023, Yu and Wang, 2023). The gene's product, XRCC2 protein, is a part of the RecA/Rad51-related protein family, is known for facilitating the exchange of strands between homologous DNA molecules (a key step in the repair process) (Liu et al., 2023). In BC, the fidelity of DNA repair mechanisms is particularly crucial, as genetic mutations driving the disease are often a result of DNA repair errors (Alhmoud et al., 2020).

Beside genomic factors like as alterations in genome sequence there are epigenetic factors that have crucial role in controlling of the expression level of the genes and their function. One of these epigenetic factors is methylation pattern in the promotor region that influence the expression level of the genes. Ke as XRCC2 and consequently lead to gain of function or loss off function in this gene.

Recent advances in molecular techniques have presented new opportunities to dissect the complexities of cancer biology. mRNA expression analysis, in particular, has become a cornerstone in studying gene expression alterations in various cancers, including BC (Malone et al., 2020, Velaga and Toi, 2022). By quantifying mRNA levels, researchers can infer the activity of genes of interest and elucidate their potential involvement in tumorigenesis and progression. This approach is instrumental in validating biomarkers for cancer diagnosis and prognosis, as well as in identifying new therapeutic targets (Perron et al., 2018). On the other hand, one of the key factors in controlling the gene expression is methylation rate in promotor region of the interested genes as epigenetics factor, so beside the mRNA level, methylation rate in promotor

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region could be considered a biomarker in cancers and could be considered a prognostic, diagnostic, even therapeutic marker in BC.

Given this backdrop, the necessity of conducting comprehensive research on the XRCC2 gene's association with BC becomes apparent and valuable. The present study aims to bridge the gap in knowledge by examining the relationship between XRCC2 and BC not at the genomic and transcriptomic level but epigenetic dimensions. This will update the knowledge on the relation between XRCC2 gene and BC from a new point of view. The necessity to delve into the molecular landscape of BC is underscored by the heterogeneous nature of the disease, which impedes the efficacy of a one-size-fits-all approach to treatment. The present study is predicated on the hypothesis that XRCC2 gene methylation levels in promotor region may serve as a biomarker in BC and could offer insights into the disease's molecular phenotype. Therefore, the present study aims to provide a detailed analysis of XRCC2 methylation in BC tissues, employing Methylation-sensitive restriction enzyme digestion PCR (MSRE-PCR).

Method

Study design and setting

In this observational analytical research study employing a case-control design, the focus was on investigating the XRCC2 gene's correlation with BC from epigenetic point of view. Conducted between 2021 and 2024, this study involved the meticulous collection of specimens from Zheen International Hospital in Erbil, Iraq.

Participants

The study included adult women 18 years and older who had been diagnosed with BC through histological confirmation and had given informed consent. Patients with previous malignancies, undergoing chemotherapy or radiation therapy prior to sample collection, lacking complete medical records, or declining to participate were excluded from the study. All subjects provided informed permission before sample collection, and the Local Ethics Committee accepted the study procedure (Approval number: 05.01.2020\17). A total of 88 samples, comprising 44 normal and 44 cancerous tissue samples from the breast, were analyzed using the prevailing sampling technique.

Methylation analysis

Based on the digestion of genomic DNA using a methylation-sensitive restriction enzyme and PCR using gene-specific primers, a methylation-sensitive restriction enzyme digestion PCR (MSRE-PCR) was used to analyze the XRCC2 promoter region's DNA methylation. Thermo Scientific EpiJET Methylation Analysis Kit (MspI/HpaII) instructions were followed in determining the DNA methylation status at a particular location. After overnight 1 µg DNA digestion was made possible by methylation-sensitive restriction enzyme cleavage by the isoschizomers Epi MspI and Epi HpaII, which have varying methylation sensitivity. Gene-specific primers were then used to amplify genomic fragments found within CpG islands in the amplified samples created by PCR. Primer design was performed for gene XRCC2 using Primer3 software, and primer sequences were as follows: XRCC2-M F-5'-TTGCTGCCATGCCTTACAGA-3', R-5'- TGGATAGACCGCGTCAA-3'. The formula for calculating the percentage of methylation was followed by the manufacturer. To identify a sample as methylated, a 20% cutoff was applied.

Results and Discussion

In terms of DNA methylation, the analysis indicated a low overall methylation rate of 7% in the XRCC2 gene in tumor tissues, with no evidence of promoter methylation. This implies that

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methylation has a restricted function in controlling the expression of XRCC2 in the examined tumor tissues (Figures 1 and 2).

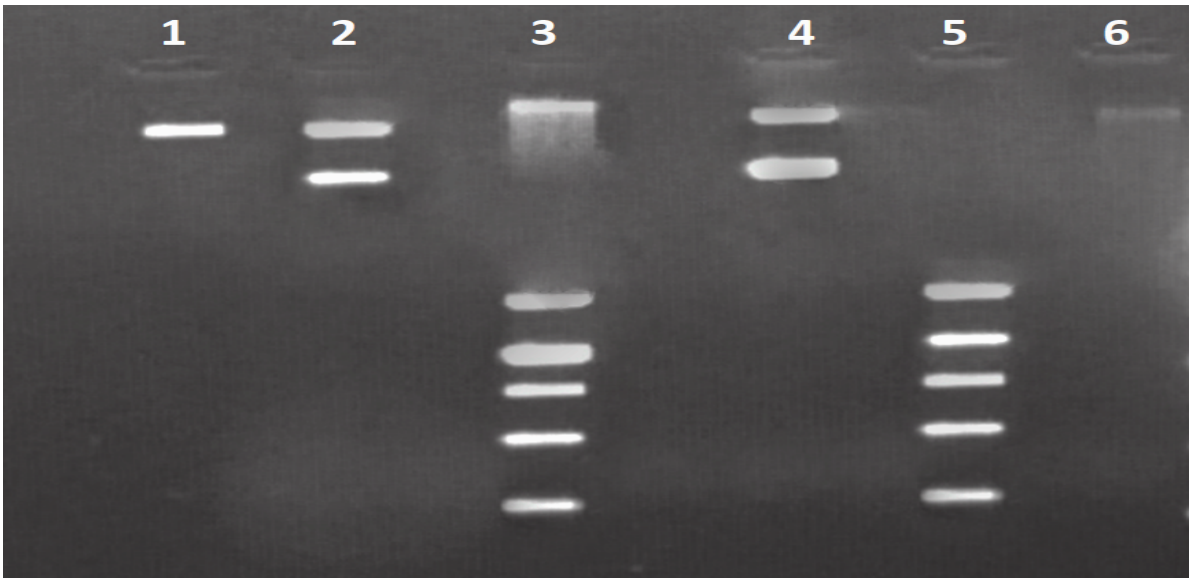

Figure 1. Digestion of genomic and control DNA by Epi MspI and Epi HpaII

Note: 1) Genomic DNA undigested; 2) contain Control pUC19/SmaI DNA CpG Methylated by Epi HpaII; 3) contain Control pUC19/SmaI DNA CpG Methylated by Epi MspI; 4) Genomic DNA and plasmid control undigested; 5) contain Control pUC19/SmaI DNA Unmethylated by Epi MspI; 6) Genomic DNA undigested

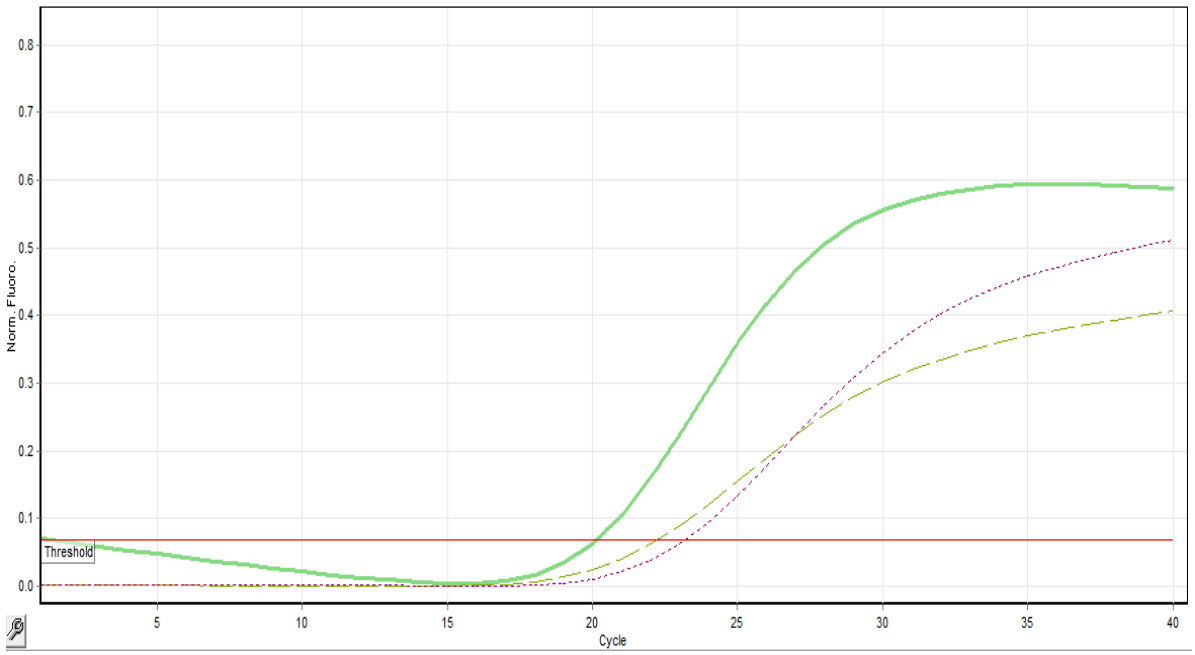

Figure 2. Quantitative XRCC2 methylation status

Note: The Green curve is “Undigested DNA”, the Doted line is “Digested with Epi HpaII” DNA, and the dashed line is Digested with Epi MspI” DNA. The ct difference between the sample digested with MspI and HpaII was calculated to be significant and less than 4.7 (partially methylated). Finally, using the formula, 7% of the XRCC2 genes were found to be methylated.

Breast cancer stands as the prevalent form of cancer in women and a primary contributor to female mortality rates (Siegel et al., 2024). Studies have shown that XRCC2 gene polymorphisms can impact BC susceptibility (Wei-Yu et al., 2011). Additionally, meta-analyses

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have highlighted the significance of genetic variability in DNA repair genes like XRCC2 in BC risk (Yu and Wang, 2023).

studies have demonstrated how methylation changes correlate with the formation of breast cancer and can be used to differentiate normal and benign breast cells from cancerous cells. A study by Elsheikh *et al.*, which characterized 880 human breast carcinomas, suggested that changes in histone acetylation and methylation patterns might represent an early sign of breast cancer (Elsheikh et al,. 2009). There is also evidence that the degree of methylation change is indicative of more aggressive metastatic breast cancer cells, compared to breast cancer cells that are less metastatic. Rodenhiser *et al.* compared the methylation status of upstream promoter region associated with genes from multiple pathways in a highly metastatic breast cancer cell line, MDA-MB-468LN, as compared to that in a less metastatic cell line, MDA-MB-468GFB. They observed hyper-methylation and hypo-methylation of genes that could be involved in carcinogenesis as well as in metastasis. Interestingly, the methylation status of 20-30% of genes differed between the two cell lines (Rodenhiser et al,. 2008).

Kluźniak et al. found that 52 of 54 cervical cancer cases exhibited hypermethylation in the XRCC2 gene's promoter region. This alteration reduces gene expression, compromising XRCC2's tumor-suppressive role in homologous recombination repair. High EZH2 levels contribute to the epigenetic silencing of RAD51 paralogs like XRCC2, weakening homologous recombination repair (Zeidler et al., 2005, Kluźniak et al., 2019)

Paulíková et al. linked XRCC2 promoter hypermethylation to severe grade III-IV toxicity in cervical cancer patients post-radiation, suggesting its potential as a predictive marker for late damage (Paulíková et al., 2013). The cancer risk associated with XRCC2 loss is partly due to increased EZH2 production, which methylates the XRCC2 promoter, leading to gene repression. This results in decreased homologous recombination repair and increased EZH2 levels, observed between 40%-75% in breast cancer, with a 7.5-fold rise in EZH2 mRNA expression (Hridy et al., 2020).

Given the significant associations observed between XRCC2 gene as an important player in DNA repair process and BC, this gene merits consideration for inclusion in genetic screening programs for at-risk populations. Such screening could potentially aid in early detection and personalized therapeutic strategies, improving patient outcomes.

The current study found a 7% methylation rate for the XRCC2 gene in tumor tissue, with no indication of methylation in the XRCC2 promoter region. The study's findings are not consistent with some previous researches that has shown DNA methylation to be a potential biomarker for assessing BC risk, allowing for the implementation of personalized screening and risk-reducing strategies. However, the specific role of the XRCC2 gene in BC is not well-established, and further research is needed to fully understand its potential implications (Ennour-Idrissi et al., 2020).

Conclusion:

In conclusion, in the present study discrepancies in the literature regarding the role of XRCC2 in BC highlight the complexity of its function, as some studies report a hypermethylation of XRCC2 gene promotor region opposite to our study. However, conflicting data and variability in study designs necessitate further research to fully elucidate the role of XRCC2 in BC carcinogenesis.

Despite the limited role of methylation in regulating XRCC2 expression within the studied samples, the existence of hypermethylations in XRCC2 and their predicted functional impact suggests a complex interplay between epigenetic alterations and breast cancer risk.

Recommendations

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Further investigations are recommended to elucidate the inconsistent findings regarding XRCC2 methylation in BC across different studies. Additionally, functional studies are warranted to understand the mechanistic role of XRCC2 in tumor development and progression. Larger, more diverse cohorts should be examined to validate the pathogenicity of the identified XRCC2 methylation pattern and to explore the association between XRCC2 promoter methylation pattern and BC.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article belongs to the authors.

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References:

Abiola, S. A., Ben-Chioma, A. E., Fidelis, B. G., Aloy, S. C. & Elekima, I. (2024). Epigenetic

Modulation in Breast Cancer: From Mechanisms to Therapeutic Interventions. *International Research Journal of Oncology,* 7(1)**,** 1-13.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2015). Molecular Biology of the Cell. New York: Garland Science.

Taylor and Francis Group, 4, 973-975

Alhmoud, J. F., Woolley, J. F., Al Moustafa, A. E. & Malki, M. I. (2020). DNA Damage/Repair Management in Cancers. *Cancers (Basel),* 12(4)**,** 1050. https://doi.org/10.3390%2Fcancers12041050

Andreassen, P. R. & Hanenberg, H. (2019). XRCC2 (X-ray repair cross complementing 2). *Atlas Genet Cytogenet Oncol Haematol,* 23(1)**,** 1-7. https://doi.org/10.4267%2F2042%2F69759

Byler, S., Goldgar, S., Heerboth, S., Leary, M., Housman, G., Moulton, K., & Sarkar, S. (2014). Genetic and epigenetic aspects of breast cancer progression and therapy. Anticancer research, 34(3), 1071-1077

Elsheikh, S. E., Green, A. R., Rakha, E. A., Powe, D. G., Ahmed, R. A., Collins, H. M., ... & Ellis, I. O. (2009). Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. Cancer research, 69(9), 3802-3809.g

Hridy, A., U., , S., , M., D., , M., , M., A., , M., S., , S., M., and Talha Bin Emran , T., B. (2020). Genetic Variations of *RAD51* and *XRCC2* Genes Increase the Risk of Colorectal Cancer in Bangladeshi Population. Asian Pac J Cancer Prev. 21(5): 1445–1451, doi: 10.31557/APJCP.2020.21.5.1445

Howlader, N. N. A. K. M., Noone, A. M., Krapcho, M., Garshell, J., Neyman, N., Altekruse, S. F., ... & Cronin, K. A. (2014). SEER cancer statistics review, 1975–2012. National Cancer Institute.

Kluźniak, W., Wokołorczyk, D., Rusak, B., Huzarski, T., Gronwald, J., Stempa, K., Rudnicka, H., Kashyap, A., Dębniak, T., Jakubowska, A., Lener, M., Szwiec, M., Tomiczek-Szwiec, J., Jarkiewicz-Tretyn, J., Cechowska, M., Domagała, P., Szymiczek, A., Bagherzadeh, M., Lubiński, J., Narod, S. A., Akbari, M. R., Cybulski, C., Bębenek, M., Godlewski, D., Gozdecka-Grodecka, S., Goździ, S., Haus, O., Janiszewska, H., Jasiówka, M., Kilar, E., Kordek, R., Kozak-Klonowska, B., Książkiewicz, G.,

https://doi.org/10.55549/zbs.1520223

Mackiewicz, A., Marczak, E., Mituś, J., Morawiec, Z., Niepsuj, S., Sibilski, R., Siołek, M., Sir, J., Surdyka, D., Synowiec, A., Szczylik, C., Uciński, R., Waśko, B., Wiśniowski, R., Byrski, T., Górski, B. & The Polish Hereditary Breast Cancer, C. (2019). Inherited variants in XRCC2 and the risk of breast cancer. *Breast Cancer Research and Treatment,* 178(3)**,** 657-663. https://doi.org/10.1007/s10549-019-05415-5

Liu, Q., Peng, Q., Zhang, B. & Tan, Y. (2023). X-ray cross-complementing family: the bridge linking DNA damage repair and cancer. *J Transl Med,* 21(1)**,** 602. https://doi.org/10.1186%2Fs12967-023-04447-2

Malone, E. R., Oliva, M., Sabatini, P. J. B., Stockley, T. L. & Siu, L. L. (2020). Molecular profiling for precision cancer therapies. *Genome Medicine,* 12(1)**,** 8. https://doi.org/10.1186/s13073-019-0703-1

Moon, J., Kitty, I., Renata, K., Qin, S., Zhao, F. & Kim, W. (2023). DNA Damage and Its Role in Cancer Therapeutics. *International Journal of Molecular Sciences,* 24(5)**,** 4741. https://doi.org/10.3390/ijms24054741

Paulíková, s., Chmelařová2, M., J. Petera1, M., Palička,V., Paulík, M. (2013). Hypermethylation of RAD51L3 and XRCC2 Genes to Predict Late Toxicity in Chemoradiotherapy-Treated Cervical Cancer Patients. Folia Biologica (Praha) 59, 240-245

Rodenhiser, D. I., Andrews, J., Kennette, W., Sadikovic, B., Mendlowitz, A., Tuck, A. B., & Chambers, A. F. (2008). Epigenetic mapping and functional analysis in a breast cancer metastasis model using whole-genome promoter tiling microarrays. Breast cancer research, 10, 1-15.

Shi, Y., Shen, M., Xu, M., Tao, M., Chen, K. & Zhu, Q. (2022). Comprehensive Analysis of the Expression and Prognosis for RAD51 Family in Human Breast Cancer. *Int J Gen Med,* 15(4925-4936. https://doi.org/10.2147%2FIJGM.S350971

Wilkinson, L. & Gathani, T. (2022). Understanding breast cancer as a global health concern. *Br J Radiol,* 95(1130)**,** 20211033. https://doi.org/10.1259%2Fbjr.20211033

Yu, J. & Wang, C. G. (2023a). Relationship between polymorphisms in homologous recombination repair genes RAD51 G172T、XRCC2 & XRCC3 and risk of breast cancer: A meta-analysis. *Front Oncol,* 13(1)**,** 1047336. https://doi.org/10.3389%2Ffonc.2023.1047336

https://doi.org/10.55549/zbs.1520223