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Polymerase Theta-mediated DNA End-Joining Repair in the Maintenance of Genome Stability

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Abstract. The DNA double-strand break (DSB) always occurs within genome. The pathways that repair the DSB are essential in keeping cell viability. This review will focus on the recent understanding of TMEJ, especially two key protein factors involved in the TMEJ repair pathway[2], DNA polymerase theta (Polθ) and DNA polymerase delta (Polδ), and discuss the potential mechanism of choosing repair pathways when there is a DNA Double Strand Break. We dissect TMEJ's unique mechanism of action, including recognition of DNA ends, microhomology search, end pairing, and DNA synthesis, by focusing on the enzyme Polθ as the central player to understand the repair pathway choice. We also discuss how BRCA-mutated cancer uses the TMEJ repair pathway to facilitate breast cancer cells' growth[3]. This review aims to provide a comprehensive overview of TMEJ's cellular functions, regulatory mechanisms, and pivotal role in BRCA-mutated breast cancer.

Keywords: DNA double-strand break; Polymerase theta-meditated end-joining; BRCA-mutated breast cancer; DNA polymerase theta; DNA polymerase delta.

1. Introduction

Double-strand break (DSB) is one of the most serious damages to DNA, which may cause chromosome rearrangement, loss of genetic information, and cell death. The DNA DSB can be caused by various reasons such as radiation, oxidation, and chemical interactions. All of these destructive factors could destroy the phosphodiester bonds in nucleotides. When the replication forks encounter bulky protein factors [1] on the DNA double-strand, breakdown of the double-strand DNA may also occur. To deal with these disruptions and avoid the death of cells, the human body has evolved different DNA repair mechanisms. In this review, we focus on a DNA DSB repair pathway, polymerase theta-mediated end-joining (TMEJ), which has not been well characterized previously. TMEJ has been considered as an important factor in maintaining genome stability, but may also be an inducer for BRCA-mutated breast cancers [2].

Two well-recognized DNA DSB repair pathways are homologous recombination (HR) and non-homologous end joining (NHEJ). The NHEJ repairs DNA DSBs by using proteins to connect the break and ligate the phosphodiester bonds. The NHEJ repair pathway is the most efficient way to fix DNA DSBs, but the ligation also causes random base pairs to be inserted into the break and destroys the genome integrity. The HR repair pathway is the opposite to the NHEJ. It consumes more energy and maintains genome integrity by using another double-strand DNA as the template and forming a D-loop with the broken DNA [3]. The TMEJ repair pathway requires coupling polymerase delta (Polδ) and polymerase theta (Polθ) for DNA DSB [4]. During the TMEJ repair, the DNA strands need to anneal 2-6 nucleotides to expose the microhomology ends for Polθ to recognize single-strand DNA at the end of the break for the TMEJ protein complex to bind to start the repair, and then the flap DNA sequence needs to be cut by the Polδ, functioning as a 3'-5' exonuclease. The Polθ synthesis will finally switch to the Polδ since Polδ is a more accurate polymerase [5]. The cooperation between Polθ and Polδ demonstrates that the TMEJ repair pathway is a precise and efficient repair pathway, which is suitable for large-scale DNA DSB repair, such as in mitotic DNA synthesis [6].

Under the condition where HR is inhibited in BRCA-mutated breast cancer, TMEJ is activated as a major repair mechanism. BRCA1 and BRCA2, are pivotal genes in maintaining genome integrity by regulating DNA repair, in particular HR. Mutations in these genes disrupt HR, leading to increased

risk of developing breast cancers. In cells lacking functional BRCA1/2, alternative DNA repair mechanisms, such as TMEJ, become crucial for cell survival. TMEJ is involved in a unique pathway of DNA repair that provides a vital alternative to HR, particularly in BRCA-mutated cancers. Translesion synthesis DNA Polθ emerges as a key player in TMEJ. Recent findings highlight a dual role of TMEJ in both promoting cancer cell growth under exgenous stresses and contributing to mutational landscape characteristics of BRCA-mutated tumors. The importance of TMEJ in these contexts suggests that TMEJ, specifically Polθ in the signaling, could be a potential therapeutic target. Inhibition of TMEJ could selectively impair the viability of cancer cells deficient in HR. In this review, we summarize recent findings highlighting the opposite roles that TMEJ may play in maintaining genome stability and in tumorigenesis resulted from TMEJ-created ‘‘genome scar‘‘ during repair of DNA DSBs.

2. TMEJ Repair Pathway

The TMEJ repair pathway utilizes two pivotal proteins Polθ and Polδ [7]. The first step of the TMEJ repair pathway is to identify the 3' single-strand DNA at the end of the double-strand DNA break after the 5‘ to 3‘ nucleotide resection through the Polθ, which is a protein around 290 kDa. Then, the Polθ will anneal 2 to 6 nucleotides until the microhomologous DNA sequence is exposed as single-strand DNA through its HelD activity *(FIG 1a)*. The binding of the Polθ paves the way for further TMEJ repair steps. Since one Polθ can only anneal one end of the DNA DSB, there need two molecules of Polθ to work together to expose both of the microhomology ends on either side of the DNA DSB. The second step in the TMEJ repair pathway is to use the 3’ to 5’ exonuclease activity of Polδ to cut the flap DNA that is exposed through Polθ annealing. Removal of the flap trimming sequence is the prerequisite for the further action of Polθ to extend the double-strand DNA after the microhomology ends on either side of the DNA strick *(FIG 1b).* The flap trimming step through Polδ clears the space for Polθ to function. Polθ will extend on the trimmed single-strand DNA sequence if the flap trimming sequence is not removed. Thus, there is a switch from Polθ to Polδ after the annealing of the double-strand DNA sequence by Polθ, and the Polδ will cut the exposed single-strand DNA sequence with its endonuclease activity [8]. Following flap trimming, the Polθ experiences its polymerase activity after the microhomology ends stick together and extend several base pairs at the position where the flap DNA sequence is cut. The first synthesis is an MH-primed synthesis and only a few base pairs are extended at the position where the flap was trimmed through Polθ. Then there is a switch from Polθ to Polδ for the processive synthesis. The processive synthesis utilizes the dual role of the Polδ as a polymerase because the Polδ is a more accurate polymerase with higher fidelity than Polθ [9] *(FIG 1b).* The combination of Polθ and Polδ demonstrates coordinated activities for the process of the TMEJ repair pathway and is further proved by the physical interaction between Polθ and Polδ. The TMEJ repair pathway provides a novel avenue for repairing DNA DSBs with minimal DNA template sequence by removing DNA template sequences without mutation. The most obvious difference between TMEJ and HR or NHEJ is that TMEJ requires Polδ endonuclease activity to flap trimming single-strand DNA sequence. As a result, the TMEJ repair pathway is error-prone and causes the loss of genetic information [10]. In conclusion, the TMEJ repair pathway demonstrates a combination of genomic stability and instability, explaining the reason why TMEJ makes DNA sequences change more frequently in the BRCA-mutated breast cancer genome.

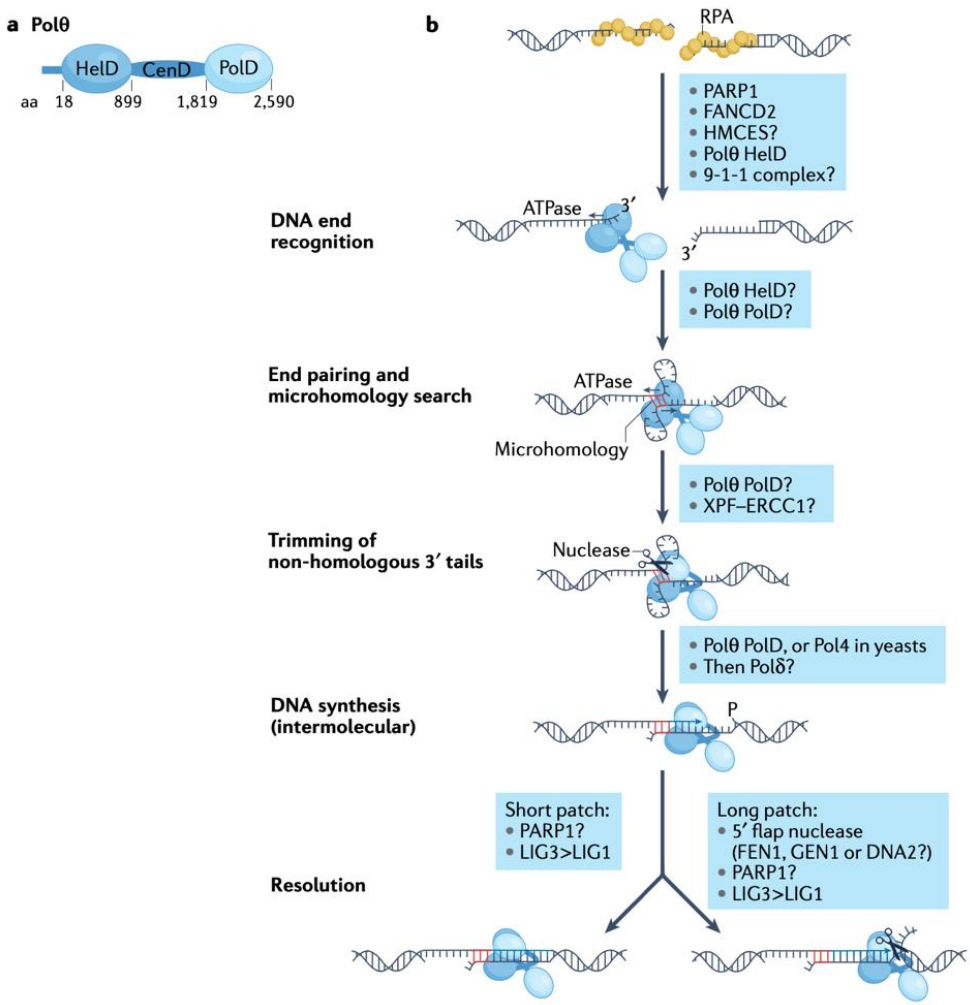

Fig. 1 [26] **The Procedures of TMEJ Repair Pathway. a.**| Structure of the DNA polymerase theta (Polθ). There are three domains in Polθ, a helicase domain (HelD) for annealing DNA sequence to the microhomology ends, a polymerase domain (PolD) for the MH-primmed synthesis, and a central domain (CenD) for connection. **b.**| Procedures of TMEJ repair pathway. RPA is a protein that binds DNA single strands. Following the recognition of the single DNA end by Polθ, annealing the DNA strand and pairing the microhomology end start. The Polδ endonuclease that cuts the trimmed ends follows. Polθ is applied for MH-primed synthesis, and then there is a switch from Polθ to Polδ for the processive synthesis.

3. The TMEJ Repair Pathway is Complementary to NHEJ or HR.

The TMEJ repair pathway is complementary to NHEJ or HR and is mainly related to the end resection at the DNA DSB [11]. The NHEJ repair pathway directly ligates the DNA DSB by inserting 1 to 3 base pairs at the breaking point [12]. The HR repair mechanism utilizes a restriction enzyme when the DNA with a break forms a D-loop to the intact DNA template [13]. TMEJ repair pathway utilizes Polθ to anneal the trimming DNA sequences and Polδ to cut the flap trimming DNA sequences with its restriction enzyme activity [14]. The accessibility of restriction enzymes during the cell cycle profoundly influences the availablility of TMEJ since restriction enzymes are only available between S and G2 phases after the CDK mediator is phosphorylated [15] (*FIG 2a*). Without the CDK mediator phosphorylation, the TMEJ repair pathway cannot be functional predominately. TMEJ is particularly adept at repairing breaks that involve complex end structures or when there are microhomologies near the break sites [16]. Although TMEJ might lead to nucleotide deletion, it provides a crucial repair option that prevents more deleterious consequences of unrepaired DSBs, such as chromosomal translocations and genomic instability. The TMEJ repair pathway distinguishes itself by functioning under cellular environments where NHEJ and HR repair pathways face limitations or fail *(FIG 2b)*,

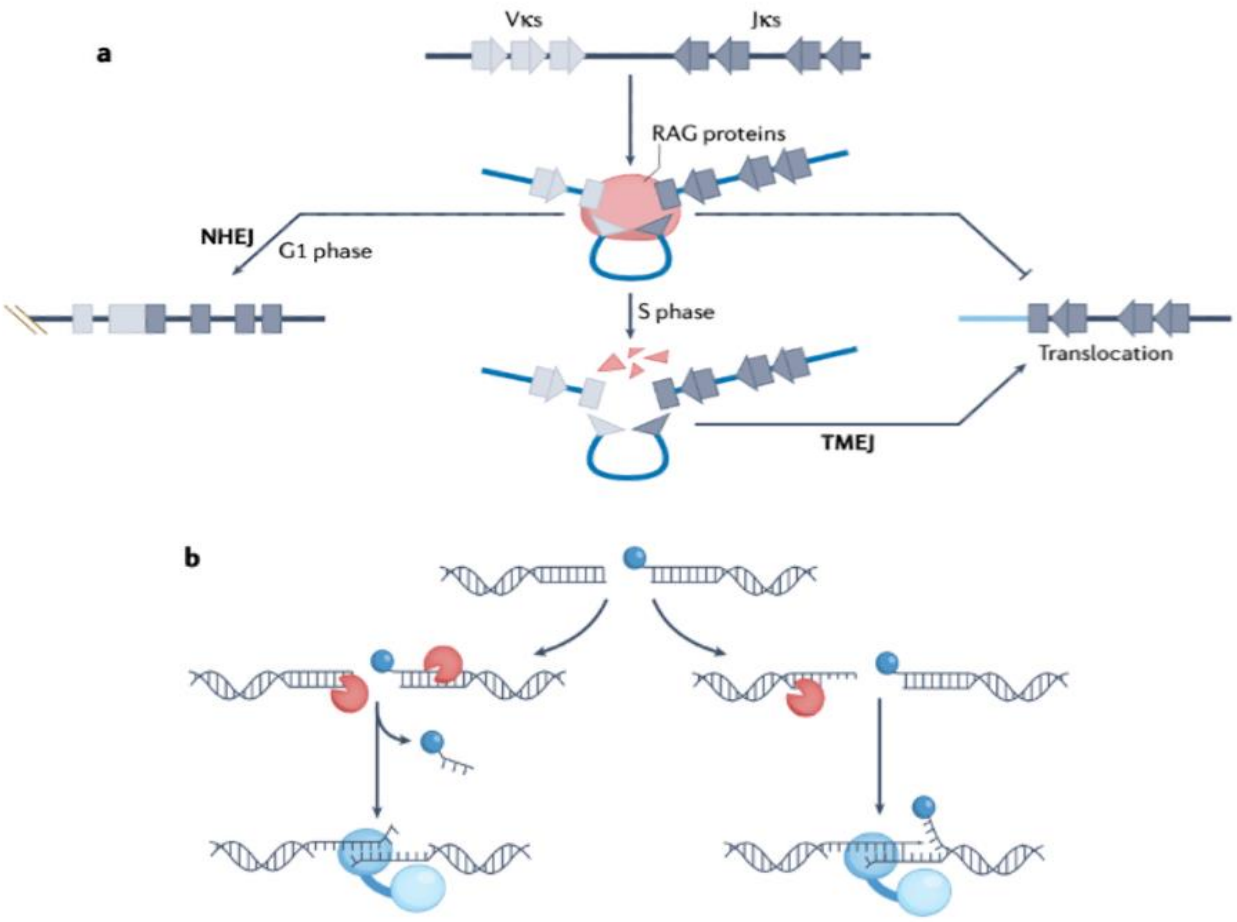

Fig. 2 [27] **The TMEJ Repair Pathway is Complementary to NHEJ or HR. a.|** NHEJ DNA repair is employed in G1 phase of the cell cycle when the restriction enzyme is unavailable. In S phase of the cell cycle, TMEJ repair pathway is favored for the translocation. **b.|** TMEJ is complementary to NHEJ repair pathway. TMEJ functions under the condition where NHEJ is inhibited by removing block protein (left) through endonuclease or using Polθ (right) for polymerase.

such as BRCA-mutated cancer or FANCJ deficiency in *Caenorhabditis elegans* [17]. In *C. elegans*, studies have shown that when the FANCJ helicase, a protein crucial for HR, is non-functional, TMEJ compensates for the loss of HR activity. This indicates that TMEJ plays a pivotal role in maintaining genomic stability in the absence of functional HR machinery [18]. Similarly, in BRCA-mutated cancers, where HR is severely compromised due to mutations in key HR genes like BRCA1 and BRCA2, TMEJ becomes the primary repair pathway [19]. The reliance on TMEJ in these cancer cells facilitates their survival and proliferation despite defective HR. Understanding this relationship is critical, as it explains why BRCA-mutated cancer cells continue to grow and resist certain therapies, highlighting the potential for targeting TMEJ in therapeutic intervention [20]. The interplay between TMEJ, NHEJ, and HR underscores the cellular strategy to maintain genomic stability under a variety of conditions. While NHEJ offers a quick fix and HR ensures high fidelity, TMEJ provides a necessary alternative that balances speed and accuracy, especially under conditions of stress or in cells with compromised repair capacity. The utilization of the TMEJ repair pathway helps cells to maintain genome stability but also contributes to the “genome scar” nature that may cause BRCA-mutated breast cancer.

4. The TMEJ repair pathway contributes to BRCA-mutated cancer

The TMEJ repair pathway is crucial in the proliferation of BRCA-mutated breast cancer cells. BRCA1 and BRCA2 are key proteins for the HR repair pathway, which brings a homologous DNA template for the precise repair of DSBs. Mutations in BRCA1 or BRCA2 disable HR, forcing cells to rely on alternative, often error-prone repair mechanisms such as TMEJ [21]. TMEJ, mediated by Polθ, repairs DSBs using microhomologies near the break sites, which frequently leads to small insertions or deletions [22]. Unlike HR, TMEJ provides a repair mechanism even in the absence of a sister chromatid. In BRCA-mutated breast cancer cells, the loss of HR capability necessitates reliance on TMEJ for DSB repair. The shift towards TMEJ is not merely a compensatory mechanism but a critical factor for the survival of these cancer cells. TMEJ allows these cells to manage DNA damage and continue proliferating despite the HR deficiency. However, this reliance on TMEJ introduces genomic instability due to the error-prone nature of the repair mechanism, leading to increased mutagenesis [23]. The characteristic of TMEJ enables tumor cells to adapt and survive. It may also accelerate tumor progression and heterogeneity. The dependence on TMEJ in BRCA-mutated cells also complicates treatment strategies, particularly the use of Poly (ADP-Ribose) Polymerase (PARP) inhibitors. PARP inhibitors exploit the HR deficiency in BRCA-mutated cells by inducing synthetic lethality. However, cells with functional TMEJ can circumvent this lethality by using TMEJ to repair DSBs. This resistance mechanism highlights the need for combined therapeutic strategies that target both PARP and Polθ [24]. Additionally, the role of TMEJ in promoting the survival of BRCA-mutated cells underscores its potential as a therapeutic target. Targeting Polθ or other components of the TMEJ pathway could sensitize BRCA-mutated cancer cells to existing treatments, such as chemotherapy and radiation, which induce DSBs. With the inhibition of TMEJ, these treatments could become more effective, as the cancer cells would have fewer pathways to repair the induced DNA damage. In conclusion, TMEJ is a vital repair pathway in BRCA-mutated breast cancer cells, compensating for the loss of HR and contributing to both the survival and aggressiveness of these cancers. The TMEJ pathway's error-prone nature facilitates genomic instability and tumor evolution, while its activity poses challenges for current therapies.

5. Conclusion

In conclusion, the TMEJ repair pathway is pivotal in keeping genomic stability, even in the context of BRCA-mutated breast cancer. The reliance on TMEJ in BRCA-mutated cancer cells, due to the loss of functional BRCA1 or BRCA2 genes, underscores its significance in facilitating cell survival and proliferation under HR deficiency. This error-prone mechanism, mediated by Polθ, introduces genomic instability, contributing to tumor progression and heterogeneity. The interplay between TMEJ, NHEJ, and HR illustrates the cellular strategies for coping with DNA damage and maintaining genomic integrity under various conditions. Despite its tendency to introduce mutations, TMEJ is able to function throughout the cell cycle, making it indispensable in certain genetic contexts, such as BRCA-mutated cancers. This dependence on TMEJ also presents therapeutic opportunities, as targeting Polθ could enhance the effectiveness of treatments like PARP inhibitors, which exploit HR deficiencies. Additionally, developing targeted therapies to inhibit TMEJ could provide new avenues for treating BRCA-mutated breast cancers, potentially improving patient outcomes by reducing therapy resistance and limiting tumor evolution. Understanding and manipulating the TMEJ pathway could thus represent a significant advancement in the fight against cancer driven by genomic instability.

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