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DNA Double-Strand Break Repair in Tumorigenesis and Anticancer Treatment

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Introduction

Although the generation of DNA Double-Strand Breaks (DSBs) is necessitated for a few processes such as immunoglobulin gene rearrangement and meiotic recombination, DSBs are among the most deleterious DNA lesions in the human genome because of their high levels of propensity to elicit genomic instability and cancer [1]. To counteract the pathological effects of DSBs, cells have evolved highly efficient repair mechanisms to minimize their detrimental effect [2]. It is envisaged that genomic instability can also be fueled by aberrant DSB repair in cancer cells, thereby rendering cells less susceptible to proliferation constraints. In addition, the formation of DSBs is an intrinsic part of DNA repair per se. Mutations in DSB repair proteins, such as BRCA1 and BRCA2, are frequently associated with an increased risk of cancer. On the other hand, the formation of DSBs also contributes to the therapeutic efficacy of many anticancer regimens using chemo- and radio-therapeutics. It is not surprising that cancer cells may acquire anticancer drug resistance through up-regulation of DSB repair pathways. Thus, a better understanding of the molecular mechanisms underpinning DSB repair will be essential for creating effective means to control cancer [3].

DNA Damage Signaling and DSB Repair

DSBs can be promptly recognized by the Ku70/Ku80 heterodimer or the MRE11-RAD50-NBS1 (MRN) complex [1]. The MRN complex helps to activate and recruit ATM, a master kinase that phosphorylates many substrates in response to DSBs [2]. For instance, CHK2 phosphorylation by ATM activates p53 and p21 leading to a G1/S checkpoint arrest. CHK2 can also inhibit the CDC25 complex to trigger an intra-S or G2/M checkpoint arrest, allocating more time for DNA repair [1]. Histone H2AX is another key substrate of ATM. In response to DSB formation, ATM phosphorylates Ser139 to form γH2AX. The latter is a loading dock for MDC1, which directly binds and recruits more ATM proteins to phosphorylate H2AX, leading to the propagation and spread of γH2AX signal for up to 1-2 Mb of DSB containing chromatin. This γH2AX signal is important for cell cycle delay and DSB repair [1].

Following the phosphorylation of MDC1, an E3 ubiquitin ligase RNF8 is recruited to DSBs, together with another E3 ligase RNF168. RNF8 promotes H2A and H2AX ubiquitination. These modifications are critical for the subsequent recruitment of BRCA1-A complex (i.e. BRCA1, RAP80, Abraxas, BRCC36, BRE and NBA1) and 53BP1, of which the recruitment of 53BP1 also requires H4K20me2 [4, 5]. The main function of BRCA1 is to facilitate 5'-end resection, and this activity of BRCA1 is antagonized by 53BP1. Therefore, the competition between BRCA1 and 53BP1 dictates the DSB repair pathway choice between Homologous Recombination (HR) and Non-Homologous End-Joining (NHEJ) [6-8]. In G1-phase cells, 53BP1 recruits the effector RIF1 to antagonize BRCA1 binding to DSBs, thereby promoting NHEJ. In S and G2-phase cells, BRCA1 precludes the accumulation of 53BP1 at DSBs, as such facilitating hMRE11-CtIP dependent end resection and HR [9].

Of the two major NHEJ sub-pathways, classical NHEJ (c-NHEJ) mediates direct rejoining of DSB ends with or without end processing [10]. This pathway begins with the binding of Ku70/Ku80 heterodimer to DSB ends and proceeds to the loading of DNA-PKcs-Artemis nucleases for end processing. The XLF-XRCC4-LIG4 complex provides the activity for end rejoining. In the absence of c-NHEJ activity, DSB end rejoining is often carried out by a less understood microhomology-mediated alternative NHEJ (a-NHEJ) pathway. It is currently conceived that the a-NHEJ pathway may play a more relevant role than c-NHEJ in genomic instability, chromosome translocation, and tumorigenesis [11]. In contrast, HR promotes error-free DSB repair by the use of a homologous repair template such as those from sister chromatids or homologous chromosomes [1]. Recognition of DSBs by the MRN complex leads to the recruitment of CDK2-activated CtIP, which is essential for the initial limited end resection activities, whereas extensive end resection is achieved through the functions of BLM-DNA2 or BLM-EXO1. The resulting 3' single-strand overhang is first coated by RPA, resulting in the activation of the ATR pathway. The recruitment of ATR-ATRIP by RPA-ssDNA leads to the autophosphorylation of ATR [12]. The subsequent loading of Rad17-RFC, 9-1-1, and TopBP1 leads to a full activation of ATR, which in turn activates CHK1 for p53 activation, CDC25 inhibition, and cell cycle checkpoint arrests. The ssDNA bound RPA is later replaced by RAD51 to form nucleoprotein filaments, which initiates strand invasion into the donor duplex [13]. This function of RAD51, which is dependent on BRCA2 and PALB2, supports several homology-directed DSB repair processes including synthesis-dependent strand annealing (SDSA), double-Holliday junction resolution, and break-induced replication.

The necessity of having efficient DSB repair is partially reflected by the strong association between DSB repair gene mutations and a high risk of cancer [1]. Mutations in *ATM, hMRE11* and *NBS1* are found in patients with Ataxia Telangiectasia (A-T), Ataxia Telangiectasia-Like Disorder (A-TLD) and Nijmegen Breakage Syndrome (NBS), respectively. Mutations in *BRCA1, BRCA2* and *PALB2* are found in familial breast cancer patients. *LIG4 or ARTEMIS* mutations associate with lymphomas, and *BLM* mutation causes Bloom Syndrome (BS).

Therapeutic Targeting of DSB Repair

The common hallmarks of cancer cells include the tendency of unlimited proliferation and defective G1 checkpoint control. Because failure to complete DNA replication can elicit mitotic catastrophe

[14,15], DNA replication has been an attractive target for therapeutic intervention in cancer-in particular, DNA replication stress caused by topoisomerase (Topo) inhibition can increase DSB formation in cancer cells and therefore promote apoptosis. Topo inhibitors commonly used in cancer therapy include Topo I inhibitors camptothecin and irinotecan, and Topo II inhibitors etoposide and doxorubicin [2,16]. A Single-Strand Break (SSB) induced by Topo I inhibition can be converted to a DSB during S phase, leading to the formation of the one-ended DSB and replication fork collapse. The repair of one-ended DSBs at the replication fork is largely dependent on HR activity. Ionizing radiation and radiomimetic drugs such as bleomycin generate both SSBs and DSBs in the genome, of which SSBs disrupt DNA replication and generate more DSBs in the process. The benefit of blocking SSB repair is clearly seen in treating BRCA1/2-deficient tumors with poly(ADP-ribose) polymerase (PARP) inhibitors [17]. PARP1/2 are important for SSB repair, and therefore PARP inhibition can cause the accumulation of DSBs to a toxic level in BRCA1/2 mutant cells owing to HR deficiency. This example underscores the importance of continuing efforts to explore new synthetic lethality strategies in anticancer therapy. DNA Interstrand Cross Links (ICLs) are another source for DNA replication-dependent formation of DSBs. The repair of ICLs involves multiple DNA repair pathways, including the Fanconi Anemia (FA) pathway, Translesion Synthesis (TLS), Nucleotide Excision Repair (NER) and HR [18]. DNA crosslinking agents are among the mainstream anticancer therapeutics [19], and include cisplatin, carboplatin, mitomycin C, melphalan and nimustine. The therapeutic efficacy of this group of compounds relies on their ability to trigger p53-dependent apoptosis or mitotic catastrophe. The fact that ICL repair is HR-dependent explains why crosslinking agents are more effective in treating FA- or HR-deficient tumor cells. In addition, DSB repair is intrinsically linked to the sensing and signaling of DSBs, therefore targeting DNA damage signaling can be also used to enhance the killing of cancer cells by DSB-inducing anticancer agents. The blocking of ATM-CHK2 or ATR-CHK1 pathway has been utilized to sensitize cancer cells to conventional DNA damaging agents. The ATM inhibitor KU55933 is being used in conjunction with IR, Topo II inhibitors and other DSB inducing drugs; for instance, ATM inhibition sensitizes p53-deficient cells to doxorubicin [15] and ATR inhibitors, VE-821 and NU6027, are effective chemotherapy sensitizers [14]. Analogously, CHK1 and CHK2 are alternative targets for this synthetic lethality [20].

Concluding Remarks

Besides surgery, which is most effective for early stage readily accessible tumors, the majority of malignancies, including those undergone surgical intervention, are routinely treated with various combinations of radio and chemotherapies. The significant obstacles for successful cancer control are the development of side effects and resistance to therapy. The recent advances in the identification of synthetic lethal relationships between DSB repair and DNA damage response pathways have made it possible to develop more effective individualized anticancer treatment strategies. Targeting specific DSB repair and DNA damage response processes in cancer cells will also minimize side effects to normal cells. Because accelerated mutation and genome alteration are invariable features of cancer cells, it is not surprising that anticancer drug resistance may arise through multiple mechanisms such as up-regulation of DSB repair, impairment of DNA damage response, and genetic reversion [14, 15]. Therefore, establishment of biomarkers that allow simultaneously monitoring the status of DSB repair and DNA damage response pathways in cancer cells during anticancer therapy will be undoubtedly essential in our continuous efforts to conquer cancer.

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