Proteins Involved in DNA Double-Strand Breaks Repair Pathways Are Essential to Prevent the Development of Cancer

Abstract

The single-stranded DNA binding (SSB) proteins play essential roles in the repair of many types of DNA damage, including double-stranded breaks (DSBs). Double-strand breaks (DSBs) are one of the severest types of DNA damage. The single-stranded binding protein is also important to maintain genome stability, since unrepaired DSBs easily induce cell death or chromosome aberrations. To maintain genome instability, cells have developed a cell-intrinsic network mechanism called DNA Damage Response (DDR) throughout most of the cell cycle. There are two main pathways of DSBs repair mechanisms, non-homologous end joining (NHEJ) and homology directed repair (HR). In this perspective, we will describe how single-stranded DNA binding proteins functions during the DSB repair pathway and their consequences for genome stability and cancer.

Keywords: Single-stranded DNA; Double-strand breaks; DNA Damage Response; Pathways; Cancer; Genetic material; Deamination; Homologous recombination; Ultraviolet; Ionizing radiation; Hypomorphic mutations; Humoral immune deficiencies; Immune response; Radio sensitivity; Adenylation

Introduction

In the last few decades, cancer research has gained remarkable insights showing that cancer is a genetic disease. Damage to genetic material is a persistent and ubiquitous threat to genomic stability. To transmit genetic information from one generation to the next, it is essential that DNA is protected from the damage caused by environmental agents and by that produced during DNA metabolism. Cells continuously encounter DNA damage from either endogenous sources including radical species as by-products of cellular metabolism or from exogenous sources. Endogenous DNA damage can lead to DNA alteration during replication; inter conversion between DNA bases generated by deamination or loss of bases following DNA modification in a process called alkylation. In addition, oxidized DNA and DNA breaks can be generated by oxygen free radicals resulting from normal cellular metabolism. Exogenous agents can be classified as air pollution, cigarette smoke, food additives, toxins, and ultraviolet rays in sunlight [1-4]. For example, physical genotoxic agents such as ionizing radiation (IR) and ultraviolet (UV) light are estimated to induce 10 [5] DNA lesions, such as chromosomal breakage (pyrimidine dimer and 6-4 photoproducts) per cell a day [4] (Figure 1). There are two types of DNA strand breaks that can occur; when the lesion is just in one of the two strands, single strand breaks occur. However, when two of these breaks are close and on opposite strands it is classified as a DNA double-strand break (DSB) [5]. DNA DSB double-strands breaks (DSBs) are an extreme threat for genome integrity because they can lead to chromosomal rearrangements or loss of genetic material. Almost all human cancers arise as a result of genomic instability that drives the carcinogenesis process.

Figure 1: DNA damage response caused by genomic stress: endogenous and exogenous.

To monitor the genome integrity, cells have developed a cell-intrinsic network mechanism called the DNA Damage response (DDR) and failure of this process often results in apoptosis or genomic instability, such as aneuploidy, deletion, or translocation.
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DNA damage response (DDR) is divided in three main steps:

I. Damage sensing;

II. Activation of transduction pathways [1];

III. DNA damage repair.

The DDR recognizes DNA lesions and initiates various downstream pathways, including cell-cycle arrest, transcriptional and post-transcriptional activation of a subset of genes associated with DNA repair, and under some circumstances triggers programmed cell death [6]. There are four distinct mechanisms of DNA DSBs repair in mammalian cells that have been classified as: non-homologous joining (NHEJ), alternative-NHEJ, single strand annealing and homologous recombination (HR). NHEJ and HR are the two major DNA DSB repair pathways [7].

Double Strand-Breaks: The Two Main Mechanism of DNA Repair

In mammal’s cell, there are two main mechanisms of double stranded-DNA breaks repair: homologous recombination (HR) and non-homologous end joining (NHEJ) [8]. Homologous recombination is an error-free repair mechanism that utilizes the genetic information contained in an undamaged sister chromatid as a repair template. Also, this mechanism predominantly operates in the late S and G2 phase of the cell cycle, since it is when the chromatid sister is available as a template and this pathway is considered a more precise to repair DSBs in DNA [9]; Otherwise, NHEJ is often error-prone pathway and involves elimination of DSBs by direct ligation of the broken ends and this mechanism can operate throughout the cell cycle. In addition, this mechanism tolerates nucleotide (nt) loss or addiction during the rejoinsingsite [10] (Figure 2).

Non-Homologous End Joining (NHEJ) Pathway

NHEJ can operate throughout the cell cycle, but it has been shown to predominate mainly during the G1 phase [11]. In mammalian cells, NHEJ initiates with a limited processing of DNA ends by the MRN complex composed of the meiotic recombination (MRE1), (RAD50) and (NBS1); also known as nibrin proteins, being considered as a central protein complex to recognise the DNA breaks [12]. When the DSBs are recognized and processed by MRN complex a signaling cascade begins which allow proteins ku-70 and ku-80 binds at the DNA ends and recruit the DNA dependent-protein kinase catalytic subunit (DNA-PK). This interaction between Ku70/80 and DNA-PK play an important rule to synapase the two DNA ends to be repaired. Also, Ku70/80 interaction seems to improve the binding equilibrium of enzymes, such as nucleases, polymerases, and ligease. These enzymes employee a sophisticated engineered machine to align a pair of ends together and perform the ligation step. Following that, once DNA-PKcs bounds to breaks ends, it seems to activate the serine/threonine kinase of this complex representing a simple’s signal transduction since it allows DNA-PKcs to phosphorylate itself causing conformational changes. Artimis is also an important enzyme to function as a 5'-3' endonuclease and these conformational changes seems to help recruiting two of known polymerases μ and λ for the NHEJ complex [13]. A complex formed by XLP, XRCC, and DNA ligase IV composes the ligation of DNA ends. The function of XRCC is stabilization ligase IV protein in cell improving its enzyme activity and efficiency of the adenylation.

The protein XLF stimulates XRCC4 binds to DNA ligase IV in the presence of divalent cationMg [14,15]. To understand this DSBs breaks pathway repair is essential since it contains many proteins that could be target to improve patients’ outcomes or eliminate cancer. During the treatment, therapeutic agent’s causes DSBs breaks in the genome as an intermediate and inhibitors could be used to block this residual process. Therefore, targeting therapy against the key signaling molecules has therapeutic implications (Figure 3).

Figure 2: Homologous recombination is an error-free repair mechanism that operates in the late S and G2 phase of the cell cycle, since it is when the chromatid sister is available. While, NHEJ can operate mainly during G1 phase or throughout the cell cycle.

Figure 3: DSBs are recognized and processed by MRN complex and a signaling cascade begins allowing proteins ku-70and ku-80 binds at the DNA ends and recruit the DNA dependent-protein kinase catalytic subunit (DNA-PK).
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Human Single-Strand DNA Binding Protein - The Key to Genomic Integrity

Single-stranded DNA (ssDNA) binding proteins play a central role in DNA during replication, recombination, DNA damage signaling, and repair in all living systems. Single-stranded-binding proteins (ssDBPs) have an efficient mechanism that limits DNA damage such as single-strand DNA breaks (ssSBs) in the cell. This is because ssDNA binding proteins (ssDBPs) have an efficient mechanism that limits DNA damage such as single-strand DNA breaks (ssSBs) in the cell.

B. Ku70 and Ku80: One of the first experimental studies involving Ku70 and Ku80 mutation showed that knockout these genes from mice display immunodeficiency, arrested B and T cell development with a significant incidence of thymic lymphomas and the cells were severely defective for recombination signal sequence. In addition to that, Ku70/-/- and Ku80/-/- mice displayed a significant growth defects and reduced size compared to wild-type mice [21]. These proteins seem to have a multifunctional role involving directly or indirectly in many cellular processes, including DSBS repair but have not been reported for Ku70/ku80 mutation in human yet. Thus, further studies focusing on Ku70 and Ku80 mechanism underlying of all the several proteins function are need, since it still remains obscure.

C. NBS1: NBS1 hypomorphic mutations and immunodeficiency are associated with human disease knowns as Nijmegen Breakage syndrome (NBS) [25]. The principal clinical manifestations of NBS patients are microencephaly, immune dysfunction, growth retardation and increased cancer predisposition. Combined cellular and humoral immune deficiencies, lymphomas showed the highest incidence among NBS patients [26]. They also present a humoral immunodeficiency presenting high variable range of agammaglobulinemia to a moderate reduction in the immune response. The most commonly reported defects are IgG and IgA [27]. In addition, NBS cultured cells are impaired to response to ionizing radiation which lead to an increased frequency of chromosomal rearrangement [28]. All these features suggest that NBS patients have defective response to DSBs.

D. RAD50: A study conducted with viable Rad50 hypomorphic mice showed these mice have profound growth retardation and most of them have developed anemia and the survival specimens developed lymphomas and leukemia as well [29]. Recently, a human disorder was associated with RAD50 gene mutation. The patient disorder was analyzed as being similar with NBS syndrome, since it is the only known patient with this disorder which shares the same clinical features, including microcephaly, growth retardation and slight ataxia. The main difference between NBS characteristics and RAD50 expression that is characterized as presenting radiosensitivity, G1/S and G2/M checkpoint defects, radio resistant DNA synthesis and increased chromosomal instability, being impaired to activate downstream signaling pathways [30]. Nevertheless, this patient was found to carry heterozygous genes for mutations.

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Conclusion

DNA double strand breaks are constantly generated in our cells either through external agents or through internal agents, including sub products from cellular metabolism. In order to maintain the genome stability cells have developed an efficient surveillance network that can detect and repair DNA breaks to protect the development of cancer or human syndromes caused by mutation of genes coding for DSB signaling and repair pathways proteins. These syndromes associated with mutations of these binding proteins involved in DSBs repair share many clinical similarities, including neurological defects, growth delay, immunodeficiency, radio sensitivity, sterility and increased cancer incidence. Increasing the knowledge on these proteins involved in DSBs repair can provided great insight into the physiological functions of DSB response proteins, which may led to rapid discoveries of new proteins that can be targeted to drugs development. Therefore, the single-stranded DNA binding (SSB) proteins is critical to prevent pathologic chromosome rearrangements and subsequent tumor development.

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Contributions

Liziara Fraporti and Tatiana Amaral Pires de Almeida contributed to: study design, analysis and interpretation, manuscript preparation, manuscript re-editing and manuscript review.

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