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DNA double-strand break repair and chromosome translocations

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ABSTRACT

Translocations are genetic aberrations that occur when a broken fragment of a chromosome is erroneously rejoined to another chromosome. The initial event in the creation of a translocation is the formation of a DNA double-strand break (DSB), which can be induced both under physiological situations, such as during the development of the immune system, or by exogenous DNA damaging agents. Two major repair pathways exist in cells that repair DSBs as they arise, namely homologous recombination, and non-homologous end-joining. In some situations these pathways can function inappropriately and rejoin ends incorrectly to produce genomic rearrangements, including translocations. Translocations have been implicated in cancer because of their ability to activate oncogenes. Due to selection at the level of the DNA, the cell, and the tissue certain forms of cancer are associated with specific translocations that can be used as a tool for diagnosis and prognosis of these cancers.

1. Introduction

A chromosomal translocation is a genomic aberration involving the rejoining of a broken chromosome fragment to another chromosome. While there need not be a net change in the amount of genetic material following this event, it can result in the disruption of genes or cause the juxtaposition of elements that disturb normal expression of the gene present at the breakpoint. This becomes critical when such translocations result in rearrangements that create genetic elements with oncogenic characteristics. In this case, it can lead to a selective advantage of cells containing these translocations with the potential for uncontrolled proliferation.

2. Clinical implications

Several documented cases of cancer exist in which translocations play an important role. One of the first described was the Philadelphia chromosome, t(9;22), found in cancer cells of patients suffering from chronic myelogenous leukemia [1]. The proto-oncogene *ABL* (*c-ABL*), a gene encoding a protein tyrosine kinase, was discovered to be located at the breakpoint on chromosome 9 [2]. *c-ABL* is highly regulated in its normal chromosomal environment, but is hyper-activated in the context of the Philadelphia chromosome translocation. Another example is Burkitt's lymphoma, where translocations involving chromosome 8 and chromosomes 2, 14 or 22 have been documented. In this case, the proto-oncogene c-*MYC* and the genes implicated in the production of antibodies (immunoglobulin (*Ig*) light (L) and heavy (*H*) chains) have been found at the breakpoints [3-5]. As a result, *MYC* is misregulated; it is abnormally overexpressed under the influence of the *Ig* gene promoters. *MYC* could increase DNA damage load by increased production of reactive oxygen species, due to its role in mitochondrial gene expression [6]. Other genes have been implicated in cancers involving translocations, for example, deregulation of the homeobox gene *HOX11* by the

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t(10;14) translocation is involved in T-cell acute lymphoblastic leukemia [7].

Over the years, many more translocations occurring in leukemias, lymphomas and solid tumors have been identified. While found mostly in hematological cancers, translocations have been implicated in cancers of mesenchymal and epithelial origin as well [8]. An online database that catalogues the occurrence of diseases with specific chromosomal aberration has been compiled (http://cgap.nci.nih.gov/Chromosomes/Mitelman).

The identification of translocations is carried out by classical karyotyping or spectral karyotyping (SKY) analysis [9]. In the clinic, certain types of chromosome translocations are used in classification of primarily haematopoeitic cancers and for the clinical prognosis of patients. For example, in an acute myeloid leukemia patient, a t(8;21) translocation is indicative of a good prognosis, that is, a positive response to treatment and long-term survival, compared to patients with a t(9;22) translocation. This information is then used to identify patients that will benefit from chemotherapy, and to determine the length and dosage of treatment [10].

3. Pathways of DNA double-strand break repair

The mechanism of the formation of translocations has been the focus of intense studies over the years. Chromosome breakage is a first step in the creation of a translocation; in its simplest form, the breakage occurs due to the formation of a DNA double-strand breaks (DSBs) in a chromosome. DSBs can be caused by both exogenous agents, such as ionizing radiation and certain chemicals, as well as by endogenous agents, including the byproducts of cellular metabolism, such as oxygen free radicals [11]. DSBs can also arise spontaneously in each S phase, for example, when a single-strand break in a parental strand is passed by a replication fork, a DSB will result [12]. Besides the pathological DSBs mentioned above, certain cell types undergo processes that require the induction of physiologically important DSBs. For example, nuclease-induced DSBs in germ cells trigger meiotic recombination that results in creation of genetic diversity. In addition, the assembly of active *Ig* and T cell receptor (*TCR*) genes as well as in *IgH* class switch recombination (CSR) occurring in the immune system requires the controlled induction of DSBs.

Repair mechanisms exist in the cell to promote the beneficial effects of the physiologically occurring DSBs and to counteract the deleterious effects of the pathological DSBs. The importance of DSB repair pathways in genome maintenance is underscored by the fact that genomic instability is a characteristic feature of cell lines and animals deficient in DSB repair pathways [13]. Proper functioning of these pathways is important in the deterrence of the illegitimate reattachment of broken chromosomes, preventing the disruption and misregulation of genes in this way. There are two mechanistically distinct methods to rejoin DNA ends, dependent on their requirement for homologous DNA sequences: homology-directed repair and non-homologous end-joining. Both can be divided in a number of subpathways that will be discussed below.

3.1. *Homologous recombination*

Homologous recombination is generally an error-free pathway of homology-directed repair. A DSB is accurately repaired by using the undamaged sister chromatid as a template for the repair of the broken sister chromatid (Fig. 1). Homologous recombination in eukaryotes is carried out by the RAD52 epistasis group of proteins, so called because they were originally identified by the genetic analysis of ionizing radiation hypersensitive *Saccharomyces cerevisae* mutants [14,15]. In human cells, the proteins in this group include the MRN (RAD50/MRE11/NBS1) complex, RAD51, the RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2, XRCC3), RAD54 and RAD54B [16]. The products of the breast cancer susceptibility genes, BRCA1 and BRCA2, are also involved in the modulation of the homologous recombination [17–19].

When a DSB is detected, the initial damage response is mediated through the MRN complex and Ataxia telangiectasia mutated protein (ATM) [20]. The resection of DNA ends is required to generate 3′ single-stranded DNA tails, which are the substrate for homologous recombination, because they are utilized for the nucleation of recombination factors on the DNA. RAD51 is an important protein at the core of homologous recombination. With the help of accessory factors, RAD51 polymerizes on the 3′ tails to create a nucleoprotein filament. After homology search, the nucleoprotein filament invades the target duplex at the site of homology to create a critical intermediate, the D-loop. This joint molecule between the broken sister chromatid and the intact sister chromatid is used as a template for DNA polymerases such that sequence information that was lost in the initial processing of the DSB end is restored. The reaction is concluded with the ligation of DNA strands and the separation of the joint molecules to yield two intact DNA copies (Fig. 1).

One of the proteins involved in the regulation of homologous recombination is the product of the breast cancer susceptibility gene, BRCA2. BRCA2 has been implicated in a mediator-type function involving multiple interactions with RAD51. BRCA2 binds to and sequesters RAD51, presumably preventing the promiscuous binding of RAD51 to DNA, that could instigate illegitimate homologous recombination within highly repetitive DNA content in the genome. However, upon DNA damage induction, RAD51 accumulates at a high local concentration into foci at the sites of damage with the help of BRCA2 [21,22]. Evidence for the involvement of Brca2 in genome stability has been provided by murine cells that express a truncated form of Brca2. These cells display impaired recombination, accumulate DNA breaks, and spontaneous chromosomal abnormalities, including translocations [23].

3.1.1. *Homologous recombination and translocations*

Translocations can occur due to an inappropriate use of recombination mechanisms [8]. In mitotic cells, specifically in the late S/G_2 phase, the template for DSB repair through homologous recombination is preferentially the sister chromatid. This guarantees that the original sequence is restored without any changes [24]. In the human genome however, the presence of highly repetitive sequences can lead to ectopic recombination, resulting in DNA rearrangements including translocations. The highly repetitive *Alu* sequences in the