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Review

Gene amplification, radiation sensitivity and DNA double-strand breaks

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ABSTRACT

DNA double-strand breaks (DSBs) are one of the main types of damage induced by ionizing radiations. Free DNA ends that are not correctly repaired can be engaged in pathways triggering gene amplification. Following gene amplification the copy number of a portion of the genome is increased, leading to an enhanced expression of the genes located in the amplified region. Gene amplification plays an important role in cancer, being one of the mechanisms of oncogene activation; in addition, it can confer resistance to chemotherapeutic agents, through the increase in the copy number of genes coding for drug targets. The presence of gene amplification can have a prognostic and a diagnostic value and can help in orienting therapy in specific tumour types. The amplified DNA is primarily produced through recombination-based pathways and can be located either within chromosomes or on extra-chromosomal acentric elements. Studies on the organization of the amplified DNA in tumour cells and in cultured drug resistant cells have suggested that a single DSB can trigger a cascade of events leading to a large number of copies of a region of the genome. In addition, it has been shown that amplified DNA is unstable, further increasing the long-term effect of the initial event. Gene amplification is a peculiar feature of transformed cells and the ability to amplify is strongly influenced by the cellular genetic background. Genes involved in DNA damage response and in DNA damage repair can play a role in controlling the amplification process, in particular, it has been shown that defects in DSB repair functions can increase the frequency of gene amplification. In this review, we will discuss the biological significance of gene amplification, together with the role of DNA DSBs and DSB repair genes in the generation of amplified DNA.

Contents

1. Introduction

DNA double-strand breaks are one of the most detrimental consequences of the exposure to ionizing radiations. When DNA breaks are not promptly and correctly rejoined, several types of chromosomal aberrations can be induced, among which gene amplification. Gene amplification is an increase in the copy number of a limited portion of the genome. It is a frequent event in tumour cells, in which it plays a major role in oncogene activation, by causing an enhancement of their expression [1]. In addition, when the amplification process engages genes involved in the response to drugs, it can lead to resistance to chemotherapeutic agents, a particularly threatening phenomenon in cancer cells [2].

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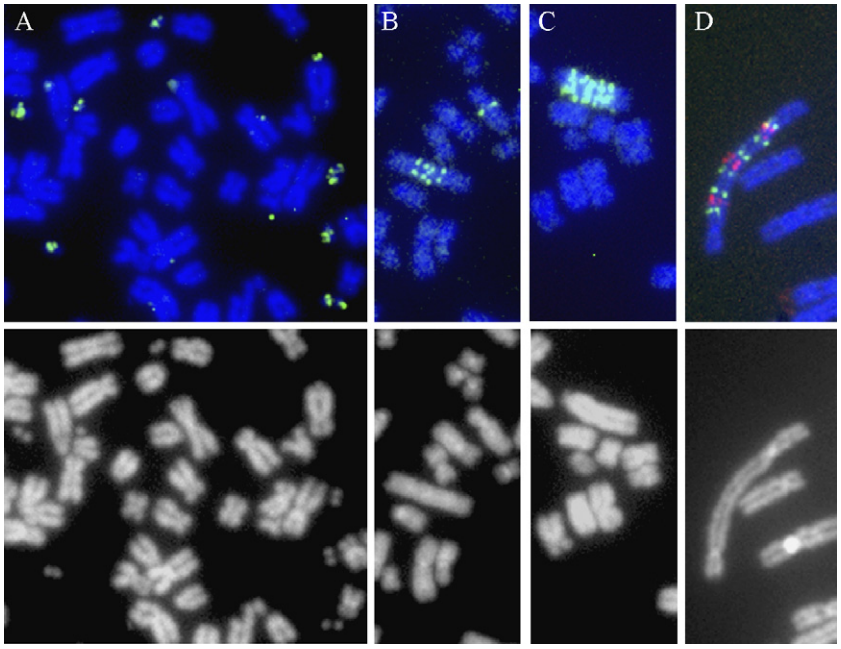

Fig. 1. Cytological organization of amplified DNA. Partial metaphases from HeLa cells resistant to MTX with amplification of the *DHFR* gene. On the upper part of the figure, the green dots correspond to the hybridization signal of the *DHFR* probe (located at 5q14.1), the red dots to the hybridization signals of a BAC containing chromosomal regions at 5q15. On the bottom part of the figure, DAPI staining of the same metaphases. (A) Example of DMs bearing the *DHFR* gene. (B) and (C) Intrachromosomal amplification of the *DHFR* gene. (B) The amplified genes are organized in an evenly spaced ladder-like structure; on the right, the single copy hybridization signal on a normal chromosome 5 is visible. (C) The amplified genes are organized in a more condensed array. (D) Double colour FISH showing the inverted repeat organization of the amplified region. The genesis of these amplified structure can be explained by BFB cycles initiated by a break downstream to the 5q15 region (see Fig. 2).

The amplified DNA can be located either on extra-chromosomal elements, known as double minutes (DMs) (Fig. 1A) or within chromosome arms (Fig. 1B–D) [3,4]. Intra-chromosomal amplified DNA is frequently localized on the same chromosome arm bearing the single copy gene (an example in Fig. 1B), giving rise to abnormally banded regions (ABRs) or homogeneously staining regions (HSRs). DMs are acentric, atelomeric circular fragments that replicate autonomously. Being acentric, DMs segregate randomly at mitosis and can be lost at cell division, unless they confer a proliferative advantage to the cells, such as when they carry amplified oncogenes or drug resistance genes. *In vitro* isolated drug resistant mutants have been a very important tool to study the organization of the amplified DNA and the mechanisms involved in gene amplification, in particular because of the possibility to study the early phases of the process. Among the drugs mostly used to select for cells in which resistance is due to amplification of the gene coding for the target protein there are: methotrexate (MTX), N-(phosphonacetyl)-L-aspartate (PALA), coformycin and hydroxyurea, which select for cells bearing amplification of *DHFR* (dihydrofolate reductase), *CAD* (carbamyl-P-synthetase, aspartate transcarbamilase, dihydro-orotase) adenosine deaminase and the M2 subunit of ribonucleotide reductase gene, respectively (reviewed in [3,5]).

Recently, the development of new techniques allowing a high resolution analysis of gene amplifications in tumours, such as the array comparative genomic hybridization (array-CGH) technique [6], has consented to better characterize the gene composition of amplicons present in different cancers, giving a chance to identify new genes and pathways playing a role in tumour development and progression [1,7]. In addition, further knowledge has been obtained on mechanisms, factors and genes controlling the process, which could contribute to identify genes to be targeted to decrease genome instability and, possibly, oncogene activation and drug resistance. In this review, we will particularly focus on the role in gene amplification of two important factors involved in the process: DNA double-strand breaks (DSBs) and DSB repair genes.

2. Biological relevance of gene amplification

Gene amplification is associated with over-expression of the amplified gene(s) and some of its most relevant biological consequences concern cancer. The use of genome-wide scanning techniques, such as array-CGH, has recently demonstrated that most solid tumours contain amplified portions of their genomes [1].

Gene amplification contributes significantly to tumourigenesis and tumour evolution, being one of the main mechanisms of proto-oncogene activation [8,9] and one of the causes of resistance to therapy [10–13]; in addition, it has been shown that amplification of the telomerase reverse transcriptase gene may be involved in the increase of telomerase activity during transformation [14–16].

The amplification of proto-oncogenes in many types of solid tumours has been a subject of innumerable studies [17,1]. *ERBB2* amplification is one of the most relevant genetic aberrations in breast cancer, being present in 10–35% of the cases [18–20]. *ERBB2* belongs to the epidermal growth factor receptor family; no mutations in its coding region have ever been detected, indicating that increased levels of expression of the gene associated with its amplification have a role in neoplastic transformation. The increased expression of ERBB2 in tumours led to the development of a therapy based on a specific antibody, Trastuzumab, directed against the extracellular domain of ERBB2 [21]. The amplification of the *ERBB2* and *myc* oncogenes in breast cancer has also acquired a prognostic value as well as *N-myc* amplification in neuroblastoma [17,22].

Amplification of the *BCR-ABL* fusion gene in chronic myeloid leukemia (CML) is one example of amplification-driven resistance to chemotherapy [12]. The *BCR-ABL* fusion gene encodes for a cytoplasmic protein with constitutive tyrosine kinase activity, which plays a major role in CML pathogenesis [23]. STI-571 can give remission in CML patients by binding to the kinase domain of ABL and inhibiting the enzymatic activity. However, in some patients treated with STI-571, amplification of *BCR-ABL* increases