Gene Amplification

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Oncogene amplification is closely linked to the pathogenesis of a broad spectrum of human malignant tumors. The amplified genes localize either to the extrachromosomal circular DNA, which has been referred to as cytogenetically visible double minutes (DMs), or submicroscopic episome, or to the chromosomal homogeneously staining region (HSR). The extrachromosomal circle from a chromosome arm can initiate gene amplification, resulting in the formation of DMs or HSR, if it had a sequence element required for replication initiation (the replication initiation region/matrix attachment region; the IR/MAR), under a genetic background that permits gene amplification.

Keywords: gene amplification ; extrachromosomal DNA ; double minutes ; micronucleus ; cancer ; genome plasticity ; chromothripsis ; gene expression ; repeat-induced gene silencing

1. Gene Amplification and the Extrachromosomal Circles in Human Cancer

The amplification of oncogenes or drug-resistant genes plays a pivotal role in human cell malignant transformation by conferring growth advantage to the cells through the overproduction of the amplified gene product. A classical cytogenetic study located the amplified genes at the extrachromosomal double minutes (DMs) or the chromosomal homogeneously staining region (HSR) [1]. DMs and HSR mutually interconvert [2][3], and share the same sequence [4]. DMs are stable extrachromosomal elements that contain circular DNA. Circularity has been suggested based on electron microscopy [5], sensitivity to radiation-mediated breakage [6], and the absence of telomeric structures [7]; this was recently re-enforced by integrating ultrastructural imaging, long-range optical mapping, and computational analysis of whole-genome sequencing [8]. In contrast, cytogenetically undetectable circular DNA has been identified in many normal and cancer cell lines and normal tissues more than three decades ago [9]. Recently, many reports have described circular extrachromosomal DNA in normal or cancer cells [10]. In general, the circles in normal cells [11][12] were smaller in size (less than 1 kbp) than those in cancer cells (1–2 Mbp) [13]. The former is referred to as extrachromosomal closed circular DNA (eccDNA), and the latter are referred to as extrachromosomal DNA (ecDNA). EcDNAs are equivalent to conventional DMs; however, the term ecDNA was recently used instead of DMs because it does not always appear as a doublet among the chromosome spread specimens. Several extensive studies that used a large number of clinical samples together with the most advanced techniques, unambiguously, reinforced the tight relationship between malignancy and the appearance of ecDNA/DMs [13][14].

It is important to note that gene expression from the same amplicon sequence is higher in the extrachromosomal context than in the chromosomal context [15] because the chromatin of extrachromosomal DNA is more favorable for gene expression [8][16]. Consistently, DMs were replicated early in the S phase, while the HSRs of the same amplicon were replicated at the end of the S phase [4]. The higher gene expression may reflect the circular nature that poses a topological constraint that favors DNA helix unwinding [8]. Alternatively, I now propose that it may reflect the plausible localization of extrachromosomal elements in the interchromosome domain (ICD)compartment, where gene expression is favored [17].

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3. From Chromosome Arm to Gene Amplification

The episome/eccDNA bearing the IR/MAR sequence was multimerized to generate larger and complex DMs/ecDNAs. The mechanism that generates an initial small circle from the chromosome arm was discussed as follows: The most plausible mechanism is chromothripsis, which is mediated by micronuclei. Chromothripsis has been suggested by cancer genomics, and it involves the abrupt fragmentation of a specific chromosome followed by re-ligation and extensive rearrangement of many fragments [18][19]. The fragmentation of a specific chromosome might occur in micronuclei [20][21] if the nuclear membrane of the micronuclei ruptures [22][23]. It has been reported that replication [24] and transcription [25] are defective in lamina-negative micronuclei. The re-ligation of the fragment produces a large number of circular molecules [26]. Among such circles, the circles with IR/MAR would be amplified as described above. A model system reproduces this process in culture [27]. It is known that human chromosomes are specifically eliminated in human-rodent hybrid cells. In such hybrids, the human chromosome was selectively incorporated into micronuclei because of the malfunctioning of the human centromere in such hybrids. Then, the micronuclear content was broken, and the human chromosome was eliminated. Importantly, there remained numerous acentric stable DMs with a mark of the human genome, that is, Alu, among stable rodent chromosomes. Such DMs are composed of a patchwork of sequences derived from multiple human chromosome regions, consistent with the structure of natural DMs/ecDNA in human cancer [28].

4. Applications of the Extrachromosomal Element-Mediated Gene Amplification

The circular plasmid DNA bearing the IR/MAR mimics gene amplification, thus providing an excellent model to study genetic plasticity associated with human malignancy. Furthermore, the system provides a novel platform for recombinant protein production, whose efficiency needs to be increased, especially in the case of biopharmaceutical production. However, this application has two major limitations. One is the cell-type dependency of the amplification efficiency ([29] our unpublished results). The problem was technically solved by amplifying the target genes on the artificial chromosome [30] [31] in the amplification-prone cells, followed by its transfer to the amplification-difficult cells by micronuclei-mediated chromosome transfer [31]. Another problem was that the amplification produced an ordered tandem repeat, which was subjected to repeat-induced gene silencing (RIGS; [32][33]). RIGS is an important cellular mechanism that heterochromatinizes the pericentric region to increase mechanical strength [34], prevent transposon spreading [35], or silence transgenes [36][37]. The problem was, at least in part, overcome by the finding that RIGS is sequence-dependent [38]. Some sequences, which included the core IR [39], the MAR, or the human genomic B-3-31 sequence, resulted in a reverse phenomenon, that is, repeat-induced gene activation (RIGA), while other sequences, which included bacterial plasmid, phage, or human transposon sequences, resulted in RIGS. Furthermore, knock-out of a histone deacetylase SIRT1 might alleviate RIGS, in combination with butyrate treatment, which inhibits another type of histone deacetylase [40]. Therefore, we are now able to amplify sequences of interest that are not subject to RIGS. We anticipate an increase in recombinant production in a gene number-dependent manner from the amplified recombinant genes.

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