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The Importance of the Specific Z-DNA Structure and Polyamines in Carcinogenesis: Fact or Fiction

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Abstract — In this work some aspects of carcinogenesis are given. The importance of the emergence of Z or H DNA structure in the gene, or in the flanking gene sequences for the gene deletion and unusual gene recombination, is discussed. Some considerations on the role of selective pressure (of polyamines, of Mg^{2+}, of the various levels of topoisomerase II, and of ATP) in the process of oncogene amplification, are given too.

Introduction

Several genetic mechanisms involved in the activation of the cellular oncogenes have been reported. They include point mutations, translocations, insertional or deletion mutagenesis, and gene amplification. In a series of recent reviews (1, 2) and papers (3–6) there are plenty of data related to the amplification of some protooncogenes in various malignant cells, and different models underlying a possible mechanism of mammalian gene amplification were given as well. There is also a number of papers linking the oncogene amplifications and clinical status of patients (7, 8, 9).

It was reported that extrachromosomal elements such as episomes and double minute chromosome(s)–DM(s), represent the most common carriers of amplified genes in human tumor cells in vivo (1, 2). It was also suggested that episomes, the submicroscopic DM's precursors, are formed by recombination within a standard replication loop.

In several recent papers (10–13), it was suggested that Z–DNA and/or cruciform structures, H-form DNA, within the inverted repeats, could be, most probably, the forms of the DNA active in recombination. Z-DNA could exist in various fragments of alternating and non-alternating purine-pyrimidine sequences frequently encountered in the mammalian (and human) introns (14, rev. 15).

The hypothesis

It could be supposed that incidental base mutations (16), or some virus gene insertions, and/or increased methylation of some DNA bases, as well as the binding of certain chemicals or proteins, could induce the emergence of Z- or H-structures at rather unpre-

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dictable sites of the DNA molecule in vivo (17, 18, 19). For the Z-DNA it was recently reported to be very susceptible to deletion both in vitro (20, 21) and in vivo (15). By an analogous mechanism in vivo, the changed (onco)gene segments (Z or Z-B-Z structure) of chromosomal DNA could be deleted by some recombination proteins, and dislodged to the karyoplasm. There it could be destroyed or stabilized by some selective pressure, in the form of either episomes or clusters (DMs?).

It was shown by experiments in vitro that methylation, or halogenation of C(5) of cytosine (15, 22), stabilize the Z-DNA structure in physiological solution. Chemical modification of DNA bases with mitomycin-C, or with AAF (N-2-acetylaminofluorene) (23, 24), also stabilize the Z structure, and result in higher potential for HIV activation and chemical cancerogenesis, respectively. It was proved that DNA methylation is frequently connected with the inactivation of the gene transcription (25), but a few cases of secondary malignancy induced by previous chemotherapy with alkylating agents were reported (26, 27).

If the Z-DNA structure is important for the (protoonco) gene deletion, for (protoonco)gene amplification in episomes (deletion–plus–episome model) (2), in DMs or in HSR, or for the deletion of a tumor suppressor gene, some inhibitors of Z-DNA stabilization could be important to abate the chances for the loss of the tumor suppressor gene, or for the (protoonco)gene amplification. Intentionally, or by chance, some potential inhibitors of Z-DNA stabilization, e.g. dealkylating agent 5-azacytidine (28, 29), or the antitumor agents, inducing the Z->B transition, such as ethidium bromide (30), actinomycin D and actinomin (31), were or are applied in the therapy of malignancies.

It was reported (1, 2) that extrachromosomal structures, such as episomes and DM(s), predominate in tumor cells in vivo. It was suggested that episomes as the carriers of some oncogenes appear during an earlier stage of tumor progression. Buttler et al (32) reported that the tumor promoter action is associated with an increased rate of protein kinase-C and ornithine decarboxylase synthesis, the enzymes which are known to be involved in the higher production of polyamines: putrescine, spermine and spermidine. It has been shown by in vitro experiments that polyamines, spermine and/or spermidine, in the presence of divalent cations (Ca^{2+}, Mg^{2+}, or putrescine) induce and stabilize toroidal and bent DNA structure (33, 34). So, in our opinion the pieces of the chromosomal DNA dislodged to karyoplasm thus, forming the episomes — after the action of the recombinant proteins (15), could be stabilized by polyamines in the same manner. For the maintenance and stabilization of these episomes (which replicate and transcribe autonomously) (1), some structures, such as spermidine or spermine, in the presence of Mg^{2+} ions, ATP, and topoisomerase II, as a selective pressure, are needed. It is known that these polyamines stabilize the bacteriophage DNA, and certain viral DNAs (33), and that their concentration is higher in malignant than in normal cells (35, 36, 37). In bacterial in vitro cultures addition of spermidine was found to double the transcription rate comparing to the cultures grown without spermidine (38).

It is well documented that the Z-DNA gets stabilization by higher superhelical densities created by the action of topoisomerase II, ATP, in physiological conditions (18, 19). It was emphasized (15) that DNA in Z structure tends to aggregate in the presence of polyamines. The work of Kim and Wang (39) showed that 'In a yeast topoisimerase double mutant TG 205 ($\triangle$ top1, top2–4) over half rDNA is present as extrachromosomal rings. The expression of Top1 or Top2 gene in the strain leads to the integration of the extrachromosomal DNA ring back into the chromosomal rDNA cluster. And it is shown that both excision of DNA rings and their integration into the chromosomes occur by recombination via homologous sequences.' Does it mean that higher level of topoisomerase II could produce higher negative super-helical densities of episomes (Z-structure) which could, in the presence of polyamines, aggregate forming clusters, DMS, or HSR region in chromosomes?

If the polyamines are important for the oncogene amplification, by the stabilization of the oncogenic transformations, then the polyamine insufficiency should result the emergence of unstable episome structures. Could this lead to some alternative chromosome abnormality (2), or to the derogation of episomes, and to the diminution of the oncogene replication rates? In other words, it should lead to the suppression of tumor propagation, or to the inhibition of tumor promotion, what has already been shown in vivo (40). Many agents used in the therapy of the various malignant alterations, both in vitro and in vivo, act as inhibitors of spermine or spermidine synthesis (41–44).

Would it be of particular interest to find out whether the application of chemotherapeutics on transformed cells, or to virus infected cell (HIV, or some other virus), in the presence of polyamine, ATP, Mg^{2+}, and various levels of topoisomerase II, in vitro could prevent the emergence of resistance, as demonstrated in bacterial system (45)?

The implication of the considerations outlined above calls for experiments that can give an answer to the question of whether the radiotherapy or chemotherapy of such systems, rich in spermine and/or spermidine, destroys more efficiently the amplified oncogenes in episomes and in DM(s) or when they are integrated in the host chromosomes? Some evidence supporting this idea has already been obtained (46).

Would the malignant cells with amplified oncogenes in HSR be more susceptible to the cytotoxic action of drugs for which topoisomerase II represent a target (47–52)? If malignant cells with the amplified oncogenes in sequence (obtained by means of unequal sister chromatid exchange) are more resistant to chemotherapy or radiotherapy, does it mean that the immunotherapy would be the most promising cure of such malignancies, as well as of malignancies whose genotype is associated with chromosomal deletions or translocations?

Conclusions

Closing this consideration, one can ask: why does the amplification of (onco)genes occur? It was shown that DNA methylation, or DNAs interaction with certain chemicals or proteins, inactivates expression of some oncogenes (53, 54). If the small quantity of their products, i.e. proteins is needed for the cell survival, the corresponding piece of DNA in Z-, or Z-B-Z-structure, or H-B-H-structure, could be excised by some single-stranded endonucleases, or SOS (15) recombinant system, deleted from chromosomal DNA, and shed into the karyoplasm, stabilized by polyamines, Mg^{2+}, ATP, and various levels topoisomerase II, to replicate, transcribe, and translate autonomously. So, in some instances the greater extent of the expression of some oncogene (55), or herpes virus transcription products in vivo (56), was found in the case of a more extensive DNA methylation. The rise of *fos* RNA induction which occurs after the action of various DNA damaging, alkylating agents was observed (57). (It is known that *fos* gene encodes a nuclear protein which bound to the transcriptional factor increases transcription from multiple promoters, as well as from promoters in the long terminal repeats of Rous sarcoma virus and human immunodeficiency virus.)

So, in order to exclude the emergence of the phenomenon of gene deletion and amplification phenomena and/or to avoid the drug resistance, or to keep the viruses in the latent forms, the inhibitors of both the alkylation i.e. of Z–DNA structure) and of polyamine synthesis should be examined in cell cultures in vitro or by in vivo experiments.

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