

Initial Phase of Epigenetic Pathways in Carcinogenesis and Mutagenesis

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Mini- Review

Epigenetic pathway is one of the major routes to carcinogenesis. A key enzyme involved in this pathway is DNA-MTase (DMT). There are evidences that carcinogens cause an increase in the activity of this enzyme. The increased activity is due to an increase in transcriptional control of the three forms of the enzyme, Dnm1, Dnm3a and Dnm3b [1]. The general function of DMTs was described by Berleth et al. [2]. The enzyme catalyses the transfer of methyl groups from 5-adenoxylmethionine (SAM) to CpG islands in key protein binding sites of DNA. The essential function of DMTs is to methylate the CpG islands in cancer suppressor genes, thus inactivating these suppressor genes and enabling the expression of cancer associated genes and thereby allowing the carcinogenic process to occur [3-5]. In normal cells, most CpG sequences are methylated but there are still 15% of which are not methylated, the CpG sequences are clustered in 20,000 regions called CpG islands which are not methylated. Aberrant methylation leads to cancer [5]. That is DMT is the key that opens the door to a major pathway leading to cancer.

DMT has a role not only in carcinogenesis but in mitogenesis as well. Yang et al. [6] showed that the action of mitogens on T-cells involves DNA methylation. In fact, epigenetic pathways may be involved in the expression of the mitogens themselves [7]. Warburton et al. [8] reported that epigenetic pathways are involved in the action of EGF on embryo cells. Hypomethylation is involved in atherosclerosis lesions, and such hypomethylation gives rise to the increased DNA synthesis in these lesions [9].

Carcinogens have been found to increase expression of DMT. Ge et al. [10] reported that Wy-14643, a carcinogen, induces hypomethylation of the c-myc oncogene which promoted the expression of c-myc. They also reported that the activity of DMT is increased by this enzyme. As we reported in our earlier paper [11], overexpression of c-myc is a characteristic of cancer. It was found that cigarette smoking causes the hypermethylation of the p16INK4-alpha gene in lung cancer patients [12]. Hammonds et al. [13] reported that in fact smoking increases expression of hepatic DMT. Carcinogen treatment increases expression of DMT as an early event in carcinogenesis. Tabish et al. [14] showed that carcinogens such as benzene, hydroquinone, styrene, carbon tetrachloride and trichloroethylene caused DNA methylation, presumably mediated by DMT. Belinsky et al. [15] showed that carcinogen treatment results in an increase in cytosine DNA-methyltransferase in target cells and this is an early event in lung cancer induced by carcinogen.

The question that has up to now been left unanswered is just how does a carcinogen induce expression of the gene coding for DMT?. It is the aim of this paper to offer a series of events that describe the mechanism whereby carcinogens can induce expression of oncogenic genes in general and DMT gene in particular.

In another of our earlier papers [16] we reviewed the extensive evidence that endocytosis and action at the lysosomal level is essential

for carcinogen action. This evidence consisted of findings that inhibition of lysosomal degradation of proteins blocked the carcinogenic process. Cathepsin inhibitors such as leupeptin, antipain, etc. were used in these studies, The same situation applies to mitogenesis induced by various growth factors [17-23]. These growth factors operate by receptors on the cell membrane. Estrogen is a carcinogen [24]. As reviewed in an earlier publication by this author [25], there is much emerging evidence that estrogen too exerts its action via receptors on the cell membrane and that endocytosis plays a vital role in the action of sex hormones. .

Thus we come to the first event when a carcinogen or a mitogen attacks its target cell: An endocytic process occurs resulting in delivery of the carcinogen-(cell membrane) receptor complex (Ca-Re) or the mitogen-receptor complex (Mi-Re) to the lysosome where the complex undergoes degradation into di and tripeptides.

What could this endocytic process, this degradation of mitogen or carcinogen receptors, play a role in the carcinogenic or mitogenic process? To answer this question we have some clues:

1. It is known that some genes are usually, in the absence of inducing agent, are repressed by protein repressors.
2. It is also known that there is specificity in the action of small peptides on proteins [26-28]. This opens the possibility that small peptides, produced on degradation of endocytosed Mi -Re or Ca-Re interact very specifically with proteins comprising gene repressors. It is quite conceivable that such an interaction can cause a change in the conformation of the repressors such that it can no longer bind to its cognate gene. That is these small peptides, acting in a very specific manner, displace the repressor from its gene. This releases the gene for expression. To summarize our proposal in equations:

$$\text{Re} + \text{Ca (or Mi)} = \text{Re-Ca (or Mi-Re)}$$

$\text{Ca-Re(or Mi-Re)} = \text{lysosomal proteases (i.e.cathepsins)} = \text{mixtures of di-and tripeptides.}$

These small peptides (with the aid of specific permeases), on formation leave the lysosomes and enter the nucleus. There they bind to various repressor proteins to liberate the Gene (Ge) for expression. That is: $\text{Ge} = \text{gene}$; $\text{Rp} = \text{repressor}$

$\text{Small peptide (formed in lysosomes as described above)} + \text{Ge-Rp} = \text{small peptide-Rp} + \text{Ge (free for expression).}$

As can be seen from our proposal, small peptides formed from degradation (by lysosomal cathepsins) of endocytosed mitogen-(or carcinogen)-receptor complexes might actually have important determinants of just which genes are to be expressed and which will remain repressed, on treatment with either a carcinogen or a non-carcinogenic mitogen (growth factor).

That leads to another concept: The identity of the genes that are derepressed (divested of repressor, thus activated) and which remain

repressed is determined by the nature of the small peptides produced in the endocytic process described above. This is determined by the following factors:

1. The nature of the proteases in the target cell. It was shown by Tanji [29]. That there are different cathepsin D isoforms and that these cathepsin D isoforms each has a different specificity. That is each isoform of cathepsin D cleaves a protein at a different peptide linkage. This will yield a different collection of small peptides formed when each isoform acts on endocytosed Mi-Re or Ca-Re complex. What determines which isoform of cathepsin D will be present in a target cell? It is not unreasonable to propose that heredity might well determine which isoform will be present in cells treated with any cytokine, that is that there is a hereditary polymorphism among cathepsins [30,31].
2. The nature of the receptors. Here again there is polymorphism such as that observed in the case of T-cell receptors [32,33]. It seems apparent that with a different substrate for the lysosomal proteases, a different set of small peptide fragments will be formed on degradation of the endocytosed receptor.

Thus one cell, cell A, equipped with one isoform of the cytokine receptor and one isoform of cathepsin in its lysosomes will produce a particular set of small peptide gene derepressors, compared with another cell (in another organism) (cell B) will produce another set of small peptide gene activators. In one case (cell A) (cell B) the activators so formed (as fragments or an endocytosed cytokine receptor) will contain all the small peptides (di- and tripeptides) needed to derepress all the oncogenes needed to initiate and develop a cancer while another cell (Cell B) may not be so endowed.

Cell A will become transformed into a clinical cancer (a neoplasia) while cell B may (depending on the nature of the small peptides produced in the endocytic process described above might) just produce a hyperplasia. Thus whether a cell will be transformed by an agent will be determined on the nature of the small peptides produced on degradation (in lysosomes) of its endocytosed agent-receptor complex, and this in turn is determined by the nature of its receptor and the nature of its lysosomal proteases (*i.e.* which receptor isoform and which cathepsin isoform is present in the target cell). This in turn is probably determined by hereditary factors.

What does all this have to do with DNA-MTase, which is the subject of this paper? From the above discussion, a possible answer emerges: In a cell destined to enter the epigenetic pathway to carcinogenesis or mitogenesis there is produced a set of small peptides which are capable of causing the repressors of the DMT gene to dissociate from the gene and thus induce the transcription of that gene or set of genes that give rise to DMT. In a cell destined to undergo carcinogenesis all the other oncogenes needed to form a cancer are activated while in a cell destined to undergo non-carcinogenic mitogenesis degradation of endocytosed agent-receptor complexes fail to produce those small peptides needed to derepress all the oncogenes (including perhaps the gene(s) coding for DMT) needed to give rise to a cancer. They produce merely a hyperplastic rather than a neoplastic situation.

This proposed mechanism therefore, in sum, answers the questions:

- 1) What is the role of endocytosis and lysosomal protease in the action of carcinogens and mitogens?
- 2) What role can small peptides produce effects on gene expression?

3) How can a carcinogen or a mitogen cause there to be increased m-RNA for DMT in treated cells?

4) How can hereditary factors determine whether an agent will produce hyperplasia in one cell (or one organism) and produce a neoplasia in another cell or even remain unchanged?

To test the above concepts, the following experiments might be performed.

Determine if in fact lysosomal protease inhibitors (including leupeptin, methylamine, antipain, etc.) inhibit production of DMT m-RNA in carcinogen treated cells.

Determine the presence of any proteins on the cell surface of cells known to be transformed by a particular agent which form a complex with the transforming agent.

Determine if there are any differences in carcinogen cell surface receptors and among the endolysosomal proteases between a cell line that is transformed compared with a cell line that is not transformed.

The above proposed series of events producing DMT might well be testable.

NOTE: That small peptides can regulate specific gene expression is not unprecedented. Indeed there have been reported some examples of specific small peptides activating specific genes [34-36]. In view of these reports it is not unreasonable to propose, as suggested by the mechanism offered in this paper, that small peptides obtained by degradation of endocytosed agent-receptor complexes could act in a similar manner: activating (by divesting genes of their repressors) genes giving rise to new cell growth, including the gene(s) coding for DMT.

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