The Regulation of Transfer RNA Methylation in Normal and Neoplastic Mammary Cells

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A number of the papers presented in this symposium have discussed the tRNA methylase activities of neoplastic cells in terms of the possible implications for altered tRNA reactivity in the translational regulation of protein synthesis. The studies carried out in our laboratory on normal and neoplastic mammary cells have led us to consider an additional implication of the altered tRNA methylase activities found in cancer cells, namely altered regulation of gene expression.

In the normal mammary epithelial cells of the C3H mouse, the activities of the specific tRNA-methylating enzymes appear to be proportional to the cellular content of tRNA (9). During development of the mammary gland in pregnancy, the total tRNA-methylating activity and the content of tRNA increase coordinately, reaching nearly maximal levels at the end of pregnancy. Six base-specific methylating enzymes have been assayed in the mammary epithelial cells, and the activity of each of these increases during the developmental period to maintain a characteristic profile of relative activities. Each of the enzymes is induced by insulin in mammary epithelial cells incubated in organ culture on chemically defined medium. As shown in the chart, incubation with insulin leads to a partially synchronized wave of DNA synthesis in the epithelial cells. This is preceded during the G1 period by increases in the cellular content of tRNA, as measured by amino acid acceptor activity, and in tRNA-methylating activity. The specific tRNA-methylating enzymes are induced in an approximately coordinate pattern during this period (9). In the postmitotic period, prolactin and insulin can induce further increases in each of the enzymes in daughter cells pretreated with insulin and hydrocortisone. In each case, the hormone-mediated increases are prevented by actinomycin D or cycloheximide, indicating that the increased activities depend upon concomitant synthesis of RNA and protein.

Comparisons of these tRNA-methylating activities with those found in spontaneous and serially transplanted mammary carcinomas in the same mouse strain (15) have yielded 4 differences: (a) the specific activity of total tRNA methylase in mammary carcinoma cells is higher than that in nonneoplastic mammary cells; (b) this increase is not associated with an increase in the intracellular content of tRNA; (c) this increase involves a marked increase in the activities of some specific enzymes, such as uridine 5-methylase, while those of other enzymes, such as cytidine 5-methylase, are not necessarily altered; (d) this distortion of the enzyme profile characteristic of the normal mammary cell is associated with the appearance of tRNA guanine 7-methylase, an enzyme found in a number of C3H mouse tissues but not detectable in normal C3H mouse mammary cells. Similar findings to these in the virus-associated tumors of the C3H mouse were also observed in a virus-independent, transplantable mammary carcinoma in the Fischer rat (13, 15).

The tRNA-methylating enzymes are induced in mammary epithelial cells, which are stimulated by insulin to pass through the G1 period and then to initiate DNA synthesis (11). A similar induction of tRNA methylases in the late G1 period of lymphocytes stimulated to divide by phytohemagglutinin has been reported by Gallo (3). One might predict from these results that other cell populations containing a larger proportion of cells proliferating through the G1 period would contain a higher induced level of tRNA methylase activity than a population of the same cell type containing predominantly differentiated cells in the G0 period and only a small proportion of proliferating cells. Such an effect might explain part of the increased tRNA methylase activity found in embryonic and neoplastic tissues in comparison to the methylase activity of the adult, differentiated counterpart. However, the differences encountered in the neoplastic mammary cells cannot be fully accounted for on this basis, since the relative increases in the specific tRNA-methylating enzymes are disproportionate. This effect could result from altered rates of enzyme turnover as well as altered rates of enzyme formation or altered regulation of enzyme activity. Regulation of tRNA-methylating enzymes does not appear to be coupled to the cellular content of tRNA in the carcinoma cells by the same relationship observed in the nonneoplastic cells. Turnover of tRNA conceivably could be increased in the mammary carcinoma cells; our studies have not yet provided evidence relating to the rates of turnover of tRNA in these cells, and the higher methylase activities could in fact be associated with increased rates of tRNA assembly. This is unlikely, however, in view of the markedly reduced cellular contents of tRNA observed in the carcinomas (15).

The most attractive explanation for the presence of tRNA guanine 7-methylase in the neoplastic cells is that the gene(s) for this enzyme is repressed in the mammary epithelial cell but becomes activated as a consequence of the neoplastic transformation. Such an explanation may apply to the formation of new methylated tRNA bases by enzymes isolated from virus-transformed cells as reported by Mittelman et al. (7) and by Hacker and Mandel (4). This interpretation is
Regulation of tRNA Methylation in Mammary Cells

Chart 1. Time course of the stimulation of total tRNA methylase activity, tRNA (measured by C-labeled aminoacyl formation), and DNA synthesis in C3H mouse midpregnancy mammary epithelial cells incubated in organ culture on medium containing insulin. Each value for DNA synthesis represents the incorporation of thymidine-3 H during the preceding 4-hr period. The methods have been previously described (9, 15). Ordinate radioactivity values should be multiplied by 100 (DNA) or 1000 (tRNA) to obtain total radioactivity.

consistent with the observation that the mammary carcinoma cells studied here form rapidly labeled nuclear RNA species (sequences) which are not detectable in the normal mammary cells by RNA-DNA hybridization-competition reactions (10, 12, 16).

These studies further emphasize the conclusion that more information will be required relating to the regulation of transcriptional and translational processes before the role of tRNA methylation in cancer cells can be understood. There is as yet little compelling evidence to indicate that an overall increase in tRNA methylase activity in itself leads to aberrant methylation of tRNA. On the contrary, it has been shown, for example, that the increased tRNA methylase enzymes in leukemia spleen tissue extracts methylated tRNA to the same extent as the less active enzyme extracts from normal spleen (1). Although new, base-specific tRNA-methylating enzymes have been detected in a number of different cancers, it is clear that not all neoplastic cell transformations involve the induction of a new tRNA methylase (2, 6). Many types of cancer cells exhibit features that represent an altered regulation of gene expression (13, 15). The rather constant feature of altered tRNA methylase activity (5, 8) is also such a manifestation of altered transcriptional control or whether aberrantly methylated tRNA is the primary mediator of altered gene expression will require further elucidation of the nature of these basic regulatory mechanisms.

REFERENCES


Discussion

Dr. Bernfield: When you compare these normal mammary glands versus the adenocarcinomas or the normal cultured glands versus those hormonally stimulated, is there any way to account for the complete major difference in cell type, fat cell in the former and epithelial cell in the latter?

Dr. Turkington: That is a very good question. What we do is...
to incubate the tissue with a crude collagenase preparation. This separates the cell types by causing the fat cells to be dislodged from the other cells and to float to the top of the tube during centrifugation. The cell pellet also contains fibroblasts with the epithelial cells. But as far as we can tell, the ratio of epithelial or carcinoma cells to fibroblasts is comparable in all the situations.

Dr. Wainfan: I wonder a bit about the possibility that normal tissue may be carrying virus. One can assume that these mice may be virus infected. In cells carrying a latent virus, and we have shown this in the bacterial system, the methylating enzymes are not quite what they are in tissue which has no virus at all.

Dr. Turkington: That is a very good point. We know that the normal cells, the preneoplastic normal cells, contain probably 2 RNA viruses. But the mode of transmission is fairly clear in these animals. That is, it is passed down through the milk. And it is possible to raise animals by foster-weaning them or foster-nursing them so that this chain of transmission is interrupted. And by all the criteria that we can use, we can't find evidence of these viruses in these cells, yet they seem to have very comparable ratios of methylating enzyme.

Dr. Magee: Were you able to compare the pattern of the tumor methylases with pregnant or lactating mammary gland?

Dr. Turkington: As far as we can tell, the patterns are the same at all these physiological levels. And when the cells differentiate during development, they simply increase all of the methylating enzymes that they have, so that the relative amounts of the activities are the same.

Dr. Magee: Wasn't there a difference in pattern between the tumor and the pregnant?

Dr. Turkington: Yes, between the tumor and the pregnant, there was, both in terms of absolute amounts and in terms of increases or decreases in specific enzymes.

Dr. Mittelman: I think Dr. Turkington's findings are even more significant histologically when one looks at the human situation. Most human tumors are derived from the ductal epithelium so that comparisons between hyperplasias of the human and carcinomas from histologic points of view may not be as meaningful. But in the mouse system, it is very clear that the carcinomas arise from the acinar tissue, so that comparisons with pregnancy are far more accurate and lend greater validity to the differences you have shown.

Dr. Adamson: Could you compare the guanine 7-methylase in the pregnant or lactating mammary gland versus the tumor.

Dr. Turkington: Yes. We couldn't find the guanine 7-methylase in the pregnant mammary gland, although we could find it in some of the other mouse organs.
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