

Cutting Edge View

Negative Feedbacks in Normal Cell Growth and Their Suppression in Tumorigenesis

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KEY WORDS

cAMP, growth, tumorigenesis, microarray, thyroid, autonomous, adenoma, feedbacks

ABBREVIATIONS

TSH thyroid stimulating hormone

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A human is composed of the order of 10^{14} cells. Most of them result from many divisions. Considering that only one in three individuals develop a cancer, any cell has an extremely low probability to result in a cancer. This low probability is explained by the fact that different alterations must be accumulated in order to escape from normal growth control: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.¹ We have shown that, in addition, the inactivation of multiple negative feedbacks, specific for activated signal transduction pathway, might also contribute to tumorigenesis.²

Over the past years, microarray analysis has brought a huge amount of data on the genes modulated in tumors. However, the interpretation of these results is complicated by the many factors that have to be taken into account, such as possible changes in cell population or differences in tumor stage. Consequently, it is of major importance to use suitable models of the normal cell under study and of its transformed derivatives. If one assumes that the initial event in the process of transformation is the activation of a given mitogenic signal transduction pathway, chronic stimulation of this pathway in primary cultures of normal cells could be used as models of this initial step. In contrast, cell lines are well on the way to full dedifferentiation and might have adapted to culture conditions, and are therefore poor models.

A role of the cAMP signaling pathway in the stimulation of growth has been demonstrated in many cell types, including somatotrophs, thyrocytes, melanocytes, ovarian follicular granulosa cells, and keratinocytes. Activating mutations of a few positive regulators of the cAMP pathway are known to induce uncontrolled growth and tumor formation. For instance, thyroid autonomous adenomas develop following constitutive activation of the thyrotropin (TSH)/cAMP pathway, generally as a consequence of a mutation of the TSH receptor, or to a lesser extent, of the Gs-alpha protein.³ In order to identify other genes involved in tumor formation, it is of pivotal importance to know which genes are normally regulated by the cAMP-pathway. Therefore, we have mimicked *in vitro* the development of the autonomous adenomas using normal human thyroid cells in primary culture treated for 90 minutes to 2 days by TSH.⁴ We then compared the gene expression patterns of the cultures obtained by microarray analysis to those of the autonomous adenomas.⁵

The results demonstrated two major points. First, the pattern of gene expression after the proliferative stimulus shows the passage of one cell program to another, with an early induction and some repression of transcription factors, followed by changes in mRNA expression of proteins involved signal transduction, followed by induction of genes corresponding to specialized functions, and changes in expression of genes involved in processes such as general metabolism, cell adhesion and cytoskeleton rearrangement. There is some temporal overlap in these types of change. As the time of TSH stimulation increases, there is a progressive convergence of gene expression with the pattern observed in autonomous adenomas, which is up to 40% at 2 days of TSH treatment. This validates the model and the previous conclusions on the pathogenesis of these tumors.³

Second, and most importantly, our results demonstrate that, when a growth signaling cascade, i.e. cAMP, is stimulated, it induces multiple negative feedback systems acting on different levels of the pathway. Among them are phosphodiesterases decreasing the cAMP levels, RGS2 inhibiting Gs, CREM inhibiting CREB, and as shown by others, GRK inhibiting the receptors. These effects are preceded by a direct activation of phosphodiesterases by PKA.⁶ Moreover, the induction of these specific negative feedbacks is complemented by the induction of other proteins inhibiting proliferation pathways (DUSP2, RGS16, PTP4A1, JUNB, IGF1BP) and the repression of SNARK, a signal transduction enzyme inhibiting biosyntheses. The induction of these proteins demonstrates a remarkable fail-safe control on the system: with these feedbacks, continuous growth stimulation has

little chance to occur. It is therefore striking that several of these inductions are downregulated or non-regulated in the autonomous adenomas. This finding is all the more interesting since only one third of the genome was explored and inactivating mutations as well as miRNA inhibition of translation and protein mislocalization are not reflected in the transcriptome. This reminds the conclusion of researchers in the field that constitutive activation of the TSH/cAMP pathway may be the cause of the adenomas but, by itself, is not sufficient: activation of the pathway must be complemented by inactivation of several negative feedbacks. The mechanism of these inactivations whether by epigenetic suppression, loss of genes by aneuploidy, promoter mutation or other is now under study.

It is tempting to hypothesize that the role of the suppression of multiple negative feedbacks could apply to other pathways and tumors. In fact, there are often more variants of the inhibiting enzymes of pathways than of the activating ones, for example more phosphodiesterases than adenylate cyclases⁷ or more DUSPs (MAP kinase phosphatases) than MAP kinases. The induction of at least one specific negative feedback protein by signal transduction pathways has been described in other systems: IKB for NFkB, SOCS for STAT, prolylhydroxylases for HIF, etc.

The negative feedbacks and therefore the role of their suppression in tumorigenesis are likely to be specific for the cell type and its signal transduction wiring. In a cell type in which cAMP negatively regulates proliferation, the suppressions we have identified would presumably result in growth inhibition. It would therefore be of great interest to search in several types of tumors for the suppression of specific negative feedbacks normally induced by the activating pathway in the cell of origin and also to search whether, during the progression of a benign tumor into malignant variant, there is a progressive loss of genes involved in negative feedbacks.

Further reading

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