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Growth and proliferation of the thyroid cell in normal physiology and in disease

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The growth and proliferation of the normal human thyroid cell is controlled by several regulating cascades. In *in vitro* cultures, it can grow in size in the presence of IGF-1 and high concentrations of insulin acting via the IGF-1 receptor and, after a pretreatment with TSH which induces insulin receptor, in the presence of physiological concentrations of insulin. The activation of either IGF-1 or insulin is sufficient, independently of the growth effect, to support the stimulation of DNA synthesis and proliferation by the different signalling cascades of TSH and cyclic AMP or growth factors.

The main physiological regulator in the thyroid cell is thyrotropin, which through the cyclic AMP cascade activates the functions of the cell (secretion) and the expression of differentiation (i.e. of specialized genes, Tg, TPO, etc) and induces cell proliferation. In human cells, TSH, through the phospholipase C Ca ++ cascade also activates the generation of H_2O_2, protein iodination and thyroid hormone synthesis. The thyroid stimulating immunoglobulins of Graves disease, TSAbs, which activate the TSH receptor, reproduce the cyclic AMP mediated effects of TSH. While cyclic AMP dependent protein kinase activation is required, in dog and human thyroid cells, for the mitogenic effect of cyclic AMP, it is not sufficient. Another intermediate is therefore necessary. At the present time, the role of EPAC guanyl nucleotide exchange protein activating Rap is investigated. The distal effect of cyclic AMP is to induce the assembly of the required complex cyclin D3-CDK4 and to cause its translocation in the nucleus. The cyclin D3 of the complex is not induced by TSH, but it has been synthesized in response to the activation through PI3 kinase of the IGF-1 receptor. The mitogenic effect of the cyclic AMP cascade requires a long term rise of intracellular cyclic AMP which explains why the short acting prostaglandin E1 has no mitogenic effect.

The best studied growth factor cascade inducing mitogenesis in dog and human thyroid cells is the EGF cascade. This cascade induces cyclin D1 and enhances the concentration of cyclin D3, but nevertheless also requires IGF-1 receptor permissive action. On the other hand, TGFβ through its receptor and a serine phosphorylation cascade, inhibits the proliferation of the thyroid cells.

Animal models of constitutive activation of such cascades have been developed. Mice expressing the adenosine A2 receptor, which behaves as a constitutive activator of adenylate cyclase in the thyroid, develop an hyperfunctioning adenoma involving the whole gland: they are hyperthyroid and have a goiter. Mice expressing the Ret PTC oncogene, which activates, somewhat as EGF, a growth factor cascade, develop a goiter and later what appears as papillary carcinomas. Mice overexpressing both human IGF-1 and IGF-1 receptor have an enlarged thyroid and some degree of autonomy (as shown by a decreased TSH serum level) but no tumor.

Human diseases illustrate applications of the concepts. Defective TSH receptors cause hypothyroidism or at least the patients require very high levels of TSH to achieve euthyroidism. Congenital (neoacquired or hereditary) constitutive activation of the TSH receptor leads to congenital hyperthyroidism. Constitutive activation of Gsα, the G protein intermediate between the TSH receptor and adenylate cyclase, cause the McCune Albright syndrome, which entails goiter and hyperthyroidism. Sporadic mutations activating the TSH receptor or Gsα in thyroid cells give rise to 80% of autonomous adenomas (even in Japanese patients!). The TSAbs of Graves disease cause hyperthyroidism and goiter. HCG, when hypersecreted in pregnancy, causes a degree of thyroid autonomy vs TSH (as shown by decreased TSH serum levels) and in patients with mutations in the TSH receptor conferring a higher than normal sensitivity to HCG, hypermesis gravidis with hyperthyroidism. Acromegaly, with its increased levels of growth hormone and consequently of local generation of IGF-1, is often accompanied by goiter and a degree of autonomy (as shown by decreased TSH serum levels).

Papillary carcinomas, in which the abnormal expression of Ret (in a constitutive form resulting from a gene translocation linking the active part of the receptor to a dimerizable domain of another protein) allows the take over of the growth factor pathways, exhibit uncontrolled growth and dedifferentiation.

On the other hand, hypersecretion of TGFβ by thyroid cells injured by the conjunction of thyroid stimulation by iodine deficiency, selenium deficiency and decreased H2O2 detoxification, and thiocyanate supply, leads to thyroid atrophy and fibrosis in rats and presumably in human endemic cretinism.

The extensive use of cell lines to study thyroid cell biochemistry has demonstrated significant differences in signalling between these models and normal thyroid cells in primary culture. Two of the classes of differences might be related:

1) the easy propensity of the cell lines to be transformed in malignant cells by oncogenes or oncogenic treatments;

2) the loss of specificity in signalling by which the normally distinct regulatory cascades (such as the cAMP cascade, the IGF-1 receptor and growth factor receptor cascades) exhibit striking cross signalling. For instance, in some WRT cells and FRTL-5 cells cyclic AMP and insulin have similar effects.

This loss of specificity is certainly favored by the very design of the procedures used for generating these cell lines. Any loss of specificity leading to a growth advantage would be selected. A similar process could take place *in vivo*. Any increase in proliferation rate entails an increased probability of genetic or epigenetic changes. Again, when favorable to growth such changes would be selected. Thus blurring of signalling specificity might be a factor in selective cell proliferation and perhaps tumorigenesis. We call this blurring "signalling entropy".