CONTROL OF PROLIFERATION AND OF THE EXPRESSION OF DIFFERENTIATION IN THYROID CELLS IN CULTURE

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In pluricellular organisms, the biology of individual cells is tightly controlled to follow the constraints and requirements of the organism as a whole. These controls are mostly expressed through extracellular signal molecules (hormones, local hormones, neurotransmitters, etc.). The cellular processes submitted to such controls can be divided into three broad categories: those dealing with the quantitative regulation of function, of growth and proliferation, and the qualitative expression of differentiation. Perversion of the two latter processes leads to tumorigenesis. The study of growth and differentiation expression has mostly used cells in culture as experimental models. In this short review, we wish to report some recent data on the control of dog thyroid cells in primary cultures and compare them to parallel results obtained with other systems and species.

The proliferation and growth of cells is controlled mainly by two classes of extracellular signal molecules, hormones and growth factors. In the case of dog thyroid cells, the following agents have been shown to directly modulate proliferation and expression of differentiation: a) hormones: thyrotrophin (TSH), insulin, somatomedin, hydrocortisone; b) growth factors: epidermal growth factor (EGF), fibroblast growth factor (FGF); c) serum; d) pharmacological probes of intracellular signal systems: phorbol esters, forskolin, cholera toxin.

Dog thyroid cells were cultured in serum-free, insulin-supplemented medium as described elsewhere. Cell proliferation has been measured by four methods: cell counting, DNA content of the culture, $^3$H thymidine incorporation into DNA, and the counting of labeled nuclei after $^3$H thymidine addition to the medium. Most results have been obtained using three of these methods. In general, treatments were generally applied after four days of culture to cells that were quiescent (1 to 2% labeled nuclei per 24 hours). Total $^3$H thymidine incorporation into DNA which is sometimes used as the only measurement, is open to the criticism that it may reflect variations in deoxyribonucleoside phosphate pool and specific activity. The expression of differentiation has been measured by iodide transport at equilibrium (C/M), iodide binding to proteins in the cells, and thyroglobulin mRNA levels.
The first question asked concerns the kinetics of proliferation after addition of various stimulating agents to the quiescent cells. Several agents, such as thyrotropin (TSH), epidermal growth factor (EGF), serum and phorbol esters, can induce, after a prereplicative phase of 15-18 hrs, the DNA synthesis of such cells, only a minority of which has proliferated in vitro before stimulation (start of DNA synthesis G1/S transition). Added alone in the presence of insulin (5 \( \mu \)g/ml), these different growth factors are sufficient to induce a wave of DNA synthesis in a part of the cell population, while the combination of TSH, EGF, and fetal calf serum (10%) induces a maximum DNA synthesis rate (>90% of the cells were labeled within 48 hrs) and an exponential proliferation (doubling time: 31 hrs) until confluence is reached. This shows that in our cultivated cells, the proliferative response to maximal stimulation is general and that under such conditions there is no distinction between a population of stem cells and a population of nonproliferating finally differentiated cells. If such a distinction is justified in vivo, it does not reflect on the behaviour of the cells in vitro. A percentage labeled mitoses curve from exponentially growing cells treated with EGF, TSH and serum together showed that the minimum time of cycle is 27 h (<G2 + M>: 3.5 h; <S>: 7 h). From these data, the G1 period is estimated to be at least 16 h which closely corresponds to the duration of the prereplicative phase of quiescent cells. This indicates that the quiescent thyroid cells are arrested in an early G1 stage rather than in an out of cycle GO phase, which supports our first conclusion.

Cell counting or DNA measurement shows that our dog thyroid cells are only able to divide four to five times. This limit is reached before confluence, which suggests an endogenous limitation. Whether this limitation reflects the in vivo situation or an artefact of the culture system is not yet known.

In the presence of insulin, TSH triggers a 30-60% cumulative labeling index within 48 h of exposure. The effect of TSH on proliferation is mimicked by cholera toxin and forskolin, whose sole known biochemical action is to activate adenylate cyclase, and by dibutyril cyclic AMP, an analog of cyclic AMP. It is, therefore, sufficient to increase cAMP levels to induce DNA synthesis in a significant part of the thyroid cell population. This clearly shows that this effect of TSH in these cells is mediated by cyclic AMP. Forskolin is particularly interesting for such work, as its effect (cyclic AMP accumulation) is very fast and disappears in a few minutes after washing of the cells. It is, therefore, possible to apply pulses of cyclic AMP accumulation or to interrupt continuous stimulation by pulses of normal resting cyclic AMP concentrations. The modulation of the length of cAMP accumulation by exposure of the cells for increasing or discontinuous periods to forskolin shows that a continuous cAMP rise for most of the prereplicative phase is necessary to support the progression in this phase until the commitment to DNA synthesis (2 hours before the start of DNA synthesis = restriction point). Interruptions in the cAMP rise as short as 2 h delayed the onset of DNA synthesis but did not postpone it by another 17 h period. These delays depend on both the moment and the duration of the cAMP interruptions. This shows that the system has a memory but that the cascade of events leading to the decision of DNA synthesis is reversible until the restriction point. Similar excessive delays in the onset of DNA synthesis are also produced by reversibly blocking protein synthesis with cycloheximide. This suggests that the induction by TSH of a commitment to DNA prereplication strictly depends on peculiarly labile cAMP-dependent events which might well be the induction by cAMP of the synthesis of a key labile protein.

The second question asked concerns the role and action of various extracellular and intracellular signal molecules in the control of proliferation and differentiation expression in our cells. After a few hours, TSH