**The Role of Growth Factors in the Regulation of Proliferation of Tracheobronchial Epithelium***

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During the last 20 years, we have seen a great expansion of knowledge in the area of biochemical and molecular regulation of cell replication. The role played by cell cycle genes and protooncogenes in controlling cell proliferation is becoming clear, biochemical pathways transducing signals from the cell membrane to the nucleus are being mapped out, and last but not least, a host of growth factors and their cognate receptors have been discovered, cloned, and sequenced, and their functions are being elucidated. From this flood of information, general principles are beginning to emerge, as well as important cell and tissue specific idiosyncrasies. This presentation focuses on a small aspect of the field of cell proliferation control, namely the regulation of proliferation of airway cells by the two peptide growth factors, transforming growth factors alpha and beta (TGFα and TGFβ), the Yin and Yang of epithelial cell proliferation.

Tissue growth factors have been studied for over 50 years. One of the first to be isolated was the epidermal growth factor, EGF.1 By now, the list of growth factors has become very large and includes TGFα and TGFβ, fibroblast growth factors, platelet-derived growth factor, endothelin, insulin-like growth factors, tumor necrosis factor, interleukins, interferons, and many others. Like hormones, growth factors bind to specific receptors in order to elicit cellular responses. All cells which express cognate receptors are targets for a given growth factor. It is the binding of the ligand to the receptor and the resulting activation of the receptor which trigger a cascade of biochemical reactions ultimately leading to transcriptional activation of genes. The only specificity inherent in the ligand lies in its ability to bind to a particular receptor. The response of the target cell is governed by the receptor, the current status of the signal transduction network to which the receptor is connected, and on the factors which regulate various levels of that network or modulate the expression of the target gene. An important principle to remember is that growth factors never act alone, but rather act in concert with other signals the cell is receiving at the time.

Transforming growth factor alpha belongs to the family of EGF-like molecules and has structural and functional homology to EGF.2 It is composed of 50 amino acids and is the proteolytic cleavage product of a 160 amino acid, transmembrane glycoprotein, pro-TGFα. In most adult tissues, TGFα is expressed at very low levels but it is highly expressed in many different tissues during embryogenesis and is upregulated during tissue regeneration and in a variety of tumors. TGFα binds to the EGF receptor, which belongs to a family of tyrosine kinase receptors which includes the insulin and the PDGF receptors. The EGF receptor has an extracellular ligand binding domain, a transmembrane and a juxtamembrane region, a cytoplasmic tyrosine kinase domain which is indispensable for signal transduction and a carboxyl terminal tail which is an autophosphorylation site exerting regulatory functions. Known substrates for the EGF receptor tyrosine kinase are second messengers and protooncogene products including PLCγ, PI3 kinase, GAP and raf.

Transforming growth factor beta belongs to a large superfamily of growth regulatory peptides including at least 5 isoforms of TGFβ, the inhibins and the Mullerian inhibiting substance family.3 The TGFβ family is highly conserved throughout evolution from Drosophila to man. There is about 70% homology between the isoforms of TGFβ. TGFβ 1, 2, and 3 are disulfide-linked homodimers of 112 amino acids. Each isoform is highly conserved between species. The TGFβs are secreted as latent complexes composed of the cleaved TGFβ proregion, the 112 amino acid bioactive region, and in some cases, a modulator protein of unknown function. Bioactive TGFβ is released by disassembly of this complex by mechanisms not clearly understood, probably involving proteases such as plasmin and possibly low pH. TGFβs 1 through 3 usually have been found to have similar activity and potency, but their expression is differentially regulated and varies with cell type. Virtually all tissues express at least one isoform of TGFβ (and the receptors). Originally, three types of TGFβ-specific binding proteins were described. However, only type I and II have been shown to be involved in TGFβ signalling. The TGFβ receptors do not belong to the family of tyrosine kinase receptors. They have as yet not been cloned, and the biochemical mechanisms through which they function are not understood. TGFβs are multifunctional tissue regulators involved in a great number of physiologic and pathophysiologic processes including embryogenesis, wound healing, inflammation, immune functions, and carcinogenesis. Besides regulation of proliferation and differentiation, the TGFβs are known to be important regulators of extracellular matrix synthesis and degradation.

One major research topic in our laboratory is to determine the biochemical and molecular basis for the abnormal growth behavior of transformed airway cells. During the course of these studies, we have learned about factors regulating growth of normal cells as well. The model we are using is rat tracheal epithelial (RTE) cell cultures, since they can be readily transformed by chemical carcinogens. The two most important (though not the only) features of the transformed phenotype of cultured RTE cells are (1) they have a greatly reduced requirement for exogenous growth factors, notably epidermal growth factor; and (2) they are “immortal,” i.e., they can be subcultured indefinitely; normal primary RTE cells can be passaged with difficulty only a few times. We hypothesized that the reason for the EGF independence might be that the transformed cells produce their own “EGF substitute,” TGFα, which may be acting as an autocrine growth stimulus.4 Many tumor cell lines have been reported to overexpress TGFα. Therefore, we examined mRNA extracted from normal and transformed RTE cells by north-
ern blot analysis for expression of TGFα transcripts and found that both types of cultures express TGFα message at similar levels. The TGFα message levels remained constant during the logarithmic growth and the plateau phases of culture. When we assayed conditioned media from normal and transformed RTE cultures for TGFα, we found that both types of cultures produced about 0.5 to 2.0 ng/10^6 cells/48 h as determined with a TGFα specific radioimmunoassay. A potentially very important difference between the two sets of cultures was that in media conditioned by transformed cells, TGFα concentrations continued to rise over time, while in media of normal RTE cultures, TGFα accumulation decreased with time. This was particularly dramatic when exogenous growth factors, ie, EGF and bovine pituitary extract (BPE), were deleted from the culture media. We then demonstrated that TGFα acts as an autocrine mitogenic stimulus using two approaches. We showed that neutralizing TGFα antibodies added to the cultures decreased cell proliferation and that tyrphostin, an EGF receptor tyrosine kinase inhibitor, significantly reduced DNA synthesis and cell growth. Taken together, these studies indicate that normal and transformed RTE cell cultures produce TGFα which acts as an autocrine growth factor. Cultures of transformed cells sustain a high rate of secretion at late stages of growth, when normal RTE cultures downregulate TGFα secretion. These findings could explain, at least in part, the enhanced growth capacity of transformed cells. Current studies are aimed at identifying possible abnormalities in the EGF receptors. The number and affinity of the EGF receptors expressed as the cell membrane of transformed RTE cells appear to be normal. It has been reported that in some transformed cell lines, the EGF receptors are hyperresponsive. Various functional measurements of EGF receptors will be examined.

TGFβ is known to be a potent growth inhibitor for many types of epithelial cells and to be expressed almost ubiquitously. Therefore, we decided to explore the following hypotheses: (1) that one or more of the TGFβ isoforms is an important growth restraining factor in normal RTE cells, limiting cell proliferation in plateau phase cultures; and (2) that one or more of the elements of the TGFβ system, ie, TGFβ synthesis/secretion, TGFβ activation (since it is secreted as an inactive complex, see above), TGFβ receptors and/or TGFβ responsiveness of the target cells, is defective in transformed cells. We found that normal and transformed RTE cells express TGFβ1 message equally and at consistently high levels (TGFβ2 message was not detected). However, normal cells secreted roughly 10 times as much TGFβ as transformed cells (15 ng/10^6 cells vs 1.2 ng/10^6 cells, per 72 h), suggesting some change in posttranscriptional regulation of TGFβ expression in transformed cells. In addition, we found that only normal RTE cells produced measurable amounts of active TGFβ, thus raising the possibility that the mechanisms involved in disassembling the TGFβ latency complex are inoperative in the transformed cells. To determine whether the secreted TGFβ was functioning as a significant growth inhibitor, RTE cell cultures were treated with TGFβ neutralizing antibodies at various stages of growth. We found that in cultures of normal RTE cells, addition of antibody to the media caused a burst of cell proliferation indicating that the TGFβ secreted by the cultures was indeed exerting a growth inhibitory effect. However, release of the cultures from TGFβ growth inhibition by TGFβ antibodies was variable, suggesting that the responsiveness of the cultures to the endogenous TGFβ was changing with time. It was therefore essential to examine the TGFβ receptors and the responsiveness of cultured RTE cells to the growth inhibitory effects of TGFβ. We found that the growth of early primary RTE cell cultures was greatly inhibited by addition of TGFβ to the media (IC50=0.2 pm), however TGFβ responsiveness diminished greatly in later phases of culture growth. The reason for this dramatic change in the TGFβ response of normal RTE cells is not clear and is currently under investigation. Many of the transformed cell lines had a greatly reduced TGFβ sensitivity (regardless of the state of growth), but a few were as sensitive as normal RTE cells. Examination of TGFβ membrane receptors revealed no differences in receptor expression, which could be correlated with loss of responsiveness. This suggested that the loss of TGFβ responsiveness might be due to a defect in receptor function(s) or a defect in some postreceptor signal transduction step. Taken together, these studies indicate that in cultures of normal RTE cells, an autocrine TGFβ growth regulatory system is exerting some restraint on proliferation. [However, it is not clear at the moment, exactly how effective that restraint is, since the late cultures exhibit decreased TGFβ responsiveness.] In transformed cell cultures, this growth restraining system is defective at several levels.

Our studies show that normal rat tracheal epithelial cells in culture produce the growth regulatory peptides TGFα and TGFβ and are responding to them; thus, an autocrine growth-stimulatory and growth-restraining mechanism was demonstrated. Chemically transformed, tracheal cells revealed several defects in these autocrine growth regulating systems consistent with the hypothesis that they are involved in the abnormal growth capacity of transformed cells.

REFERENCES


