Transforming Growth Factor–β: Biological Function and Chemical Structure

MICHAEL B. SPORN, ANITA B. ROBERTS,
LALAGE M. WAKEFIELD, RICHARD K. ASSOIAN

Transforming growth factor–β (TGF-β) is a multifunctional peptide that controls proliferation, differentiation, and other functions in many cell types. Many cells synthesize TGF-β and essentially all of them have specific receptors for this peptide. TGF-β regulates the actions of many other peptide growth factors and determines a positive or negative direction of their effects. Its marked ability to enhance formation of connective tissue in vivo suggests several therapeutic applications.

TRANSFORMING GROWTH FACTOR–β (TGF-β), A PEPTIDE first identified by its ability to cause phenotypic transformation of rat fibroblasts (*1, 2*), has now been shown to have numerous regulatory actions in a wide variety of both normal and neoplastic cells. Many different cells synthesize TGF-β (*1–3*), and essentially all have a specific high-affinity receptor for this peptide (*4–7*). TGF-β is thus a fundamental regulatory molecule, acting by both autocrine and paracrine mechanisms. Recent studies indicate an important role for TGF-β in cells of the immune system (*8, 9*) and connective tissue (*10–12*), as well as in epithelia (*13, 14*). TGF-β is multifunctional, since it can either stimulate or inhibit proliferation, can either stimulate or inhibit differentiation, and can either stimulate or inhibit other critical processes in cell function. Despite common nomenclature, TGF-β is chemically distinct from TGF-α (*15*) (an analog of epidermal growth factor, EGF), and has essentially no sequence homology with either TGF-α or EGF (*16*). As is true for most peptide growth factors, the basic molecular mechanism of action of TGF-β is at present unknown. Nevertheless, so many new and varied functions have now been described for TGF-β that, at present, one must consider this peptide to be a general mediator of regulation in the cell, and one of special importance for negative control of cell growth.

Effects on Cell Proliferation

The multifunctionality of TGF-β was first discovered in studies of its role in control of cell proliferation. The original studies defining TGF-β measured its ability to stimulate proliferation of NRK (normal rat kidney) fibroblasts in soft agar (*1, 17*). However, TGF-β was then shown to be essentially identical to a growth inhibitor that had been previously identified and partially characterized in monkey kidney cells (*18*) and which inhibited their proliferation by an autocrine mechanism (*5, 19*). Subsequently, inhibitory effects of TGF-β on proliferation of many cell lines, both neoplastic and nonneoplastic, in both monolayer and soft agar, have been described (*19–21*). Most recently, it has been shown that TGF-β is a strong inhibitor of proliferation in many primary or secondary cell cultures, including hepatocytes (*22, 23*), embryo fibroblasts (*24*), T and B lymphocytes (*8, 25*), keratinocytes (*14, 21*), and bronchial epithelial cells (*13*). It has long been known that serum may inhibit growth of many epithelial cells in culture, and TGF-β, which is found in high concentrations in serum (*26*) and inhibits proliferation of most epithelial cells, now appears to be a principal mediator of this effect; TGF-β is present in platelets (*26, 27*) in an amount equivalent to the better known platelet-derived growth factor, PDGF, and is released from α-granules of platelets when blood clots (*28*).

In a cell of mesenchymal origin, whether TGF-β stimulates or inhibits proliferation is a function of the entire set of growth factors operant in that cell (*20*); that is, the biological meaning of the signal generated by TGF-β binding to its receptor depends on the context of the other growth factors present. Thus, in fibroblasts transfected with a *myc* gene, TGF-β stimulates growth in soft agar in the presence of PDGF, while the identical concentrations of TGF-β inhibit growth of these same cells in the presence of EGF (*20*).

Although TGF-β is a strong inhibitor of proliferation of normal bronchial epithelial cells (*13*), keratinocytes (*14*), and hepatocytes (*22, 23*), malignant cells derived from these two sources may have lost their ability to be inhibited by this peptide (*14, 22, 29*), suggesting that loss of this negative growth control may be one mechanism contributing to their unrestrained growth. A related phenomenon is seen in A549 human lung carcinoma cells, which secrete large amounts of TGF-β in an inactive, latent form (*7, 30*); acid activation of the conditioned medium of these cells, or addition of exogenous TGF-β from platelets, results in potent inhibition of proliferation (*7*), suggesting the uncontrolled proliferation of these cells may be due to their inability to activate the latent secreted form of TGF-β.

A fundamental mechanism of the antiproliferative action of TGF-β is its ability to antagonize the mitogenic effects of other peptide growth factors. Although the original description of TGF-β involved its synergistic action with EGF (or TGF-α) to cause anchorage-independent growth of NRK cells in soft agar (*1, 17*), it antagonizes the mitogenic effects of these two peptides when NRK cells are grown in monolayer culture (*20*). Furthermore, TGF-β strongly blocks the mitogenic actions of PDGF on rat embryo fibroblasts (*24*), of EGF on *myc*-transfected fibroblasts (*20*), of fibroblast growth factor (FGF) on vascular endothelial cells (*31*), and interleukin-2 (IL-2) on T lymphocytes (*8*). In the case of T lymphocytes, it is known that synthesis of both IL-2 and TGF-β are induced when these cells are activated (*8, 16*); TGF-β may therefore

The authors are members of the Laboratory of Chemoprevention, Division of Cancer Etiology, National Cancer Institute, Bethesda, MD 20892.

be acting as a feedback control on excessive clonal expansion. Although the mechanism of these antiproliferative effects is not fully understood, it is clear from studies with retinoic acid that TGF-β acts through pathways distinct from those by which PDGF and EGF act (*32*), and that the signals generated from the TGF-β receptor often block those of many other peptides that promote a proliferative state in the cell.

Effects on Cell Differentiation

In some instances the above effects of TGF-β on cell proliferation are coupled with an effect on differentiation. Again, both stimulatory and inhibitory results have been found. Thus, TGF-β induces squamous differentiation and production of cornified envelopes in bronchial epithelial cells (*13*); this is an example of terminal differentiation induced by TGF-β. TGF-β may also act to stabilize a differentiated state, by blocking further cell proliferation. Thus it inhibits the proliferative effects of insulin and hydrocortisone on kidney epithelial cells, but does not inhibit protein synthesis induced by these hormones (*33*); the net result of this action is to stabilize the differentiated state induced by the hormones and to cause cellular hypertrophy. In contrast, the antiproliferative effects of TGF-β may in some instances be coupled to an inhibition of further cell differentiation, as in the case of B lymphocytes (*25*); the differentiation of these cells to a state where they secrete immunoglobulins is blocked by TGF-β.

However, effects of TGF-β on differentiation are not always coupled with an inhibitory effect on proliferation. For example, the well-known differentiation of 3T3 fibroblasts into adipocytes (*34*), induced by insulin and glucocorticoids, is strongly blocked by TGF-β (*35*); in this case TGF-β does not block the induction of mitosis and proliferation induced by the above two hormones. Thus, a variety of significant effects of TGF-β on cell differentiation have been reported, and these effects may or may not be coupled to an antiproliferative effect. In a manner similar to its actions on cell proliferation, TGF-β regulates or directs the nature of the cellular response to various other differentiation signals.

Other Regulatory Functions of TGF-β

There are numerous effects of TGF-β on cell function that are not directly related to its actions on proliferation or differentiation. Like many growth factors, TGF-β stimulates glucose (*36*) and amino acid transport (*37, 38*), as well as glycolysis (*37, 38*), in fibroblasts. A more specific action is its marked enhancement of collagen and fibronectin formation in these cells (*11, 12*); in vivo, this leads to a fibrotic response at the local site of injection (*10, 12*). Its stimulatory effects on function of other cell types are diverse and cell-specific. As examples, TGF-β enhances prostaglandin release and mobilization of calcium in calvaria in organ culture (*39*), while in nondividing ovarian granulosa cells it markedly potentiates the ability of follicle-stimulating hormone (FSH) to induce the production of progesterone and estrogen (*40*) and it alters the formation of receptors for luteinizing hormone (LH) induced by FSH (*41*).

Several inhibitory effects of TGF-β on cell function are also known, such as its ability to block natural killer (NK) function in lymphocytes (*9*) and its suppression of steroidogenesis induced in adrenal cells by adrenocorticotropin (ACTH) (*31*). The ability of interleukin-2 to up-regulate its own receptor in activated T lymphocytes is also inhibited by TGF-β (*8*). TGF-β thus again directs or regulates the nature of cellular responses to other signals, especially those induced by other peptide growth factors or hormones.

Structure of TGF-β and Its Gene

TGF-β is a dimer (molecular weight 25,000) that, on reduction, yields two identical chains of 112 amino acids (*16, 27, 42–44*); only the dimer is biologically active. TGF-β is highly stable under acidic conditions; full activity is retained in 1*M* acetic acid at 95°C, and acid-ethanol extraction is a practical way to isolate TGF-β from many tissues. The presence of 18 half-cystine residues in each dimeric molecule undoubtedly contributes to this stability. The total amino acid sequences of human (*16*) and mouse (*45*) TGF-β are known from their cloned complementary DNA's (cDNA's) and show a remarkable identity, differing only in a single amino acid. Bovine (*43, 46*) and porcine TGF-β (*47*) have also been partially sequenced and are identical to the human molecule, as far as is known. The cDNA for TGF-β shows that the monomer of 112 amino acids is derived by proteolytic cleavage from a precursor protein of 391 amino acids (*16, 45*), of which the processed monomer represents the carboxyl terminal portion; the precursor is also highly conserved from man to mouse and contains the sequence Arg-Gly-Asp-Leu, a critical part of the cell attachment domain of fibronectin (*48*).

Recently, several other peptides have been shown to be very similar, if not identical, to TGF-β. These include a growth inhibitor isolated from the conditioned medium of monkey kidney cells (BSC-1) (*18, 19*) and a cartilage-inducing peptide (CIF-A) isolated from bovine bone (*46, 49*); the yield of the TGF-β–like peptide from bone is almost 100-fold greater than that found in most soft tissues, suggesting an important role in the physiology of bone.

Peptides Related to TGF-β

Another regulatory peptide that has been found to have structural homology with TGF-β is inhibin (*50*), a specific and potent polypeptide inhibitor of the pituitary secretion of FSH. Inhibin has been isolated from ovarian follicular fluid and its cDNA cloned (*51*); it is a heterodimer and a product of a gene family that includes TGF-β. Because of its suppression of FSH, inhibin has potential to be used as a contraceptive in both males and females; the possibility of making chimeric heterodimers with TGF-β has also been suggested (*51*). However, it is already clear that inhibin acts through its own receptor, and that TGF-β does not function as an inhibin in cell culture (*52*).

Yet another peptide that belongs to the TGF-β family is Müllerian inhibitory substance (MIS), produced by the testis and responsible for the regression of the Müllerian ducts (anlagen of the female reproductive system) in the male embryo (*53, 54*); MIS has been shown to inhibit the growth of human ovarian cancer in nude mice (*55*). The human and bovine genes for MIS have recently been cloned and the peptide itself is homologous, in terms of the location of its cysteine residues, to TGF-β (*56*). It is of interest that both inhibin and MIS, like TGF-β, have strong growth-inhibitory activities.

Receptor for TGF-β

TGF-β binds to a specific cell membrane receptor of high affinity (K_d = 1 to 60 p*M*) that is found in essentially all cells, normal or malignant, epithelial or mesenchymal, including cells of hematopoietic origin such as lymphocytes (*4–7*). There is essentially no cross-reactivity between TGF-β and receptors for other growth factors, nor do other growth factors bind to the TGF-β receptor (*4*). Unlike receptors for EGF and PDGF, that are extensively down-regulated

by exposure to high ligand concentrations or following malignant transformation of cells, the receptor for TGF-β is relatively resistant to extensive down-regulation by its ligand (*4, 57*). The TGF-β receptor is a large (approximately 500 to 600 kD) molecule with two subunits linked by disulfide bonds (*58, 59*); it appears to have no tyrosine kinase activity (*58*), unlike the receptors for most other growth factors.

Potential Therapeutic Importance of TGF-β

The action of TGF-β in vivo, especially at the local site of application to connective tissue, is strongly anabolic and leads to fibrosis and angiogenesis (*10, 12*), suggesting that TGF-β may have practical applications in repair of tissue injury caused by trauma, burns, surgery, or debility in the aged. Platelets (*27*), macrophages (*60*), and lymphocytes (*8*), cellular elements that play an essential role in tissue repair (*61, 62*), all release significant amounts of TGF-β when activated. The ability of TGF-β to promote collagen formation may also relate to a metabolic condition such as osteoporosis, in which inadequate formation of collagen or other components of the bone matrix may contribute to pathogenesis (*63*). Still another area of potential therapeutic importance is the suppressive action of TGF-β on both T and B lymphocytes, suggesting practical applications as an antiinflammatory or immunosuppressive agent. It has not yet been possible to explore many potential applications of TGF-β because of the lack of availability of the relatively large amounts of pure peptide needed for studies in vivo. However, when recombinant material does become available, it may be expected that many new studies will become possible, enabling a broader evaluation of the physiological and therapeutic actions of this versatile molecule.

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