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Review

Development of TGF-β Resistance During Malignant Progression

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Transforming growth factor-β (TGF-β) is the prototypical multifunctional cytokine, participating in the regulation of vital cellular activities such as proliferation and differentiation as well as a number of basic physiological functions. The effects of TGF-β are critically dependent on the expression and distribution of a family of TGF-β receptors, the TGF-β types I, II, and III. It is now known that a wide variety of human pathology can be caused by aberrant expression and function of these receptors. The coding sequence of the type II receptor (RII) appears to render it uniquely susceptible to DNA replication errors in the course of normal cell division. By virtue of its key role in the regulation of cell proliferation, TGF-β RII should be considered as a tumor suppressor gene. High levels of mutation in the TGF-β RII gene have been observed in a wide range of primarily epithelial malignancies, including colon and gastric cancer. It appears likely that mutation of the TGF-β RII gene may be a very critical step in the pathway of carcinogenesis.

Key words : TGF-β, TGF-β receptors, TGF-β resistance, Mutation, Carcinogenesis

INTRODUCTION

Transforming growth factor-β (TGF-β) has been one of the most intensely investigated molecules in the past decade (Massagué, 1990; Massagué *et al.*, 1994; Roberts and Sporn, 1990). Named for its ability to sustain anchorage-independent growth of fibroblasts in soft agar, TGF-β was first successfully isolated from a variety of tissues. With the development of specific antibodies which recognize the different subtypes of TGF-β, its widespread distribution in nearly every human tissue has been demonstrated. Highest concentrations have been observed in the extracellular matrix of bone and alpha granules of human platelets. During embryonic development, TGF-β appears to play an important part in organogenesis and tissue induction and differentiation. In the mature organism, TGF-β plays principal roles in inflammation and repair mechanisms (Roberts and Sporn, 1990). Clinical trials have already demonstrated efficacy of locally applied TGF-β in accelerating soft tissue and bone healing. Transgenic animal technology has led to the creation of a TGF-β null phenotype mouse which rapidly develops severe diffuse inflammatory syndrome affecting primarily the heart and lungs and which leads uniformLy to death within an average of three to four weeks (Kulkarni *et al.*, 1993; Shull *et al.*, 1992). Abnormalities or excessive response of the TGF-β ligand or receptor system most likely contribute significantly to the pathology witnessed in numerous human diseases, including arthritis, ahterosclerosis, and glome- rulonephritis. Due to its potent ability to inhibit cell proliferation, speculation occured early on that defects in the TGF-β receptor system might represent a common step in the development of malignancy. This prediction has born fruit in spectacular fashion within the past two years. Mutations in the TGF-β type II receptor were first identified in human gastric carcinoma (Park *et al.*, 1994). Less than a year later, it was reported that a subpopulation of colon cancers possessing flawed DNA repair mechanisms demonstrated a very high frequency of mutations in the type II receptor. Similar mutations have been identified in liver cancer, small cell lung cancer, and bone and soft tissue malignancies.

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TGF-β superfamily

The extended TGF-β superfamily currently consists of at least twenty-five different members, all peptides which share one unique structural motif:seven-precisely positioned cysteine residues which interact to create a "cycteine knot" (Massagué, 1990). The proteins possess an amino terminal signal peptide which directs the fully translated product to the cell membrane. In addition, the initial amino acid sequence contains a propeptide segment of variable length which is cleaved at a dibasic (RXXR) site to yield the functionally active carboxy terminal fragment.

The initial ligands to be characterized, TGF-β1, TGF-β2, and TGF-β3 now represent members of a well-defined subfamily within the larger TGF-β superfamily. TGF-β1, TGF-β2 and TGF-β3 appear to occur in all mammalian organisms (Roberts and Sporn, 1990). Although the patten of expression of any individual isoform is distinct in the developing organism, in vitro each ligand is typically interchangeable in various assays. Most likely, the unique appearance of each isoform during embryonic development represents a designated role for each isoform, however, at this time, clearly distinct functions for the three isoforms have been difficult to establish.

The activin subfamily consists two peptides, inhibin βA and inhibin βB, which can either homodimerize to form activin A and activin B, respectively, or heterodimerize with another peptide, inhibin alpha, to form inhibin A or inhibin B, respectively. The activins and inhibins have opposing effects on gonadal and pineal differentiation (Roberts and Sporn, 1990).

The third subfamily is the *dpp/Vg*-related subfamily and consists of a variety of molecules that have been found to share approximately 80 percent sequence homology within the subfamily and approximately 60 percent homology when compared with the TGF-β subfamily. Members include dpp, or decapentaplegic peptide, which mediates segmentation and limb patterning in Drosophila, Vg1 which invokes axial formation and mesodermal induction in early Xenopus development, several bone morphogenic proteins (BMP 2~8) which appear to play significant roles in development of the bony skeleton. Additional members of this group include 60A from Drosophila, several growth and differentiation factors (GDF-1, GDF-3, GDF-9), nodal, dorsal, inhibin alpha, and Mullerian inhibitory substance.

TGF-β receptor superfamily

The manifold functions of TGF-β are mediated through specific cell surface receptors (Massagué, 1990; Roberts and Sporn, 1990). TGF-β has been found to bind with varying affinity to many cell-surface associated molecules. These include high affinity receptors characterized by a Kd of approximately 5-200 and low affinity receptors with a Kd greater than 1 nM (Lin *et al.*, 1992). The high affinity receptors appear to be the ones responsible for mediating most if not all specific intracellular effects of TGF-β, whereas the function of the low affinity receptors has not been definitely established. Three classes of high-affinity receptors have been identified and named according to their apparent size on electrophoretic gels, type I (53 kD), type II (75 kD), and type III (also called betaglycan, 200~400 kD).

The first receptor to be successfully cloned using a powerful COS cell expression system was the type II receptor (Lin *et al.*, 1992). Two distinct receptors, an activin type II and a TGF-β type II receptor, were originally isolated. Since that time, several other activin type II receptors along with splice-variants have been recognized. The extracellular domains of the different type II receptors are highly variable with only 14 percent sequence homology and account for variability with regards to ligand binding. The extracellular portion is relatively rich in cysteine residues, and one particular cysteine-rich motif located near the cell membrane is highly conserved among members. There is a single membrane spanning segment. The intracellular portion of the receptors is dominated by a serine-threonine kinase domain which shares 41 percent homology with other type II receptors. The serine-threonine kinase is constitutively active and functions to autophosphorylate residues on its own intracellular region as well as transphosphorylate the type I receptor (Chen *et al.*, 1995). Transphosphorylation of the type I receptor appears to be the activation step which allows signal transduction to proceed.

Partial sequencing of the type II receptors allowed subsequent cloning of the type I receptors by exploiting sequence similarity within the kinase domain and PCR technology (Franzén *et al.*, 1993; He *et al.*, 1993). Several type I receptors have been identified, including ALK-5(R4) which binds TGF-β, ALK-2 (R1) and ALK-4 (R3) which are both activin receptors. The type I receptors are a structurally related but distinct subfamily from the type II receptors. They also possess a highly conserved cysteine-rich segment in the extracellular region, and the intracellular region is again dominated by a serine-threonine kinase which shares significant sequence homology with the type II receptor kinase. The type I receptor, however, is incapable of binding ligand independent of the type II receptor, and its intracellular portion requires transphosphorylation by the type II receptor to become active (Bassing *et al.*, 1994; Brand and schneider, 1995; Capocasale *et al.*, 1995; Cárcamo *et al.*, 1995; Chen and Derynck, 1994; Chen *et al.*, 1995; Wieser *et al.*, 1993; Wieser and Massagué, 1995; Wieser *et al.*, 1992; Wieser *et al.*, 1994). Activation of the type I receptor kinase appears to be required for downstream signaling to proceed.

The final class of TGF-β receptor is the type III receptor, also referred to as betaglycan (Wang *et al.*, 1991). In many TGF-β responsive cell lines, this appears to be the most highly represented receptor type on the cell membrane with approximately 10,000 copies per cell (López-Casilla *et al.*, 1991). Its structural features are distinct from either the type I or type II receptors and consists of a larger extracellular region which binds ligand and an abbreviated intracellular region without measurable kinase activity. Endoglin is a related type III receptor which exists on

endothelial cells, kidney mesangial cells, and macrophages (López-Casilla *et al.*, 1993). Whereas betaglycan binds to TGF-β1, TGF-β2, and TGF-β3 with nearly equal affinity (TGF-β2 with slightly greater affinity), endoglin binds only TGF-β1 and TGF-β2 and does not appear to bind TGF-β3 to any appreciable extent.

Receptor-mediated signal transduction

The specific identity of the type II receptor appears to determine ligand specificity, so that the TGF-β type II receptor is capable of binding only TGF-β and not activin, however each type II receptor is capable of interacting with multiple type I receptors, for example the TGF-β type II receptor associates with both ALK5/R4 and TSR-I. It is the type I receptor that now appears to determine which cellular response to TGF-β is triggered, i.e. inhibition of cell proliferation or transcriptional activation of designated genes for cell differentiation, synthesis of extracellular matrix, etc.

Most experimental investigation of the interaction between type I and type II receptor has utilized the mink lung epithelial cell, Mv1Lu for which TGF-β receptors, and its responses have been particularly well defined (Laiho *et al.*, 1991a; Laiho *et al.*, 1991b; Moustakas *et al.*, 1993). The type II receptor serine/threonine kinase is constitutively active and autophosphorylates the intracellular domain of the type II receptor (Lin *et al.*, 1992). Kinase activity is not modulated by ligand binding. Binding of TGF-β to the type II receptor does, however, allow cooperative binding to the type I receptor. In the absence of type II receptor, the type I receptor is incapable of independently binding to TGF-β. Association between the type I and type II receptor leads to transphosphorylation of the type I receptor GS domain by the type II receptor kinase and is a requirement for activating the kinase activity of the type I receptor (Wrana *et al.*, 1994). It is activation of the type I receptor that is most directly correlated with intracellular signaling (Kawabata *et al.*, 1995).

The TGF-β type III receptor plays no direct role in signaling but instead appears to enhance ligand binding to the type II receptor, particularly by TGF-β2. Despite possessing a rudimentary intracellular domain without observable kinase activity, the type III receptor binds all isoforms of TGF-β equally well to its large extracellular domain. This is in contrast to the type II receptor which binds to the TGF-β2 isoform with significantly lower affinity than to TGF-β1 or TGF-β3. Cells lacking the type III receptor demonstrate minimal responses to TGF-β2. Transfection into these cells of functional type III receptor leads to TGF-β2 responsiveness. Similarly, increasing levels of the type III receptor in cells already expressing normal levels leads to enhanced responsiveness to all TGF-β isoforms (Henis *et al.*, 1994; López-Casilla *et al.*, 1993).

Downstream signal transduction pathways

The first clues to downstream signaling pathways from the TGF-β family serine/ threonine kinase receptors came from genetic studies in Drosophila demonstrating that effects of weak alleles of the TGF-β family ligand decapentaplegic (Dpp) could be enhanced by mutations in a maternally exprressed gene called mothers against dpp (Mad) (Raftery *et al.*, 1996), and that Mad could partially rescue the dpp null phenotype. Studies in C. elegans resulted in identification of 3 genes, sma-2, sma-3, and sma-4, homologous to Mad, which have a mutant phenotype similar to that of the mutant daf-4 (a type II receptor serine-threonine kinase) (Savage *et al.*, 1996). Further studies clearly implicated these Mad and sma homologous in downstream signaling from the receptors (Newfeld *et al.*, 1996) (Fig. 1).

The vertebrate homologous of the Mad and sma genes have been called Smads. To date, 9 or 10 different Smad genes have been decribed which fall into three distinct functional sets: signal-transducing, receptor-activated SMADs, which includes Smads 1, 2, 3, 5, 8, and 9; a single common mediator SMAD, Smad4/DPC4, and inhibitory SMADS, Smads 6 and 7 and the Xenopus protein, Xsmad8 (Heldin *et al.*, 1997).

The present model for downstream signaling that is emerging from these studies is that 1) receptor-activated SMADs bind to and are phosphorylated on two C-terminal serine residues in their MH2 domain by the type I receptor kinase (Souchelnytskyi *et al.*, 1997); 2) the phosphorylated, pathway-specific SMADs then form a heteromeric complex in the cytoplasm with the common mediator (Lagna *et al.*, 1996), Smad4; 3) the phosphorylated SMAD/Smad4 complex is then translocated to the nucleus where it participates in a transcriptional complex and mediates activation of

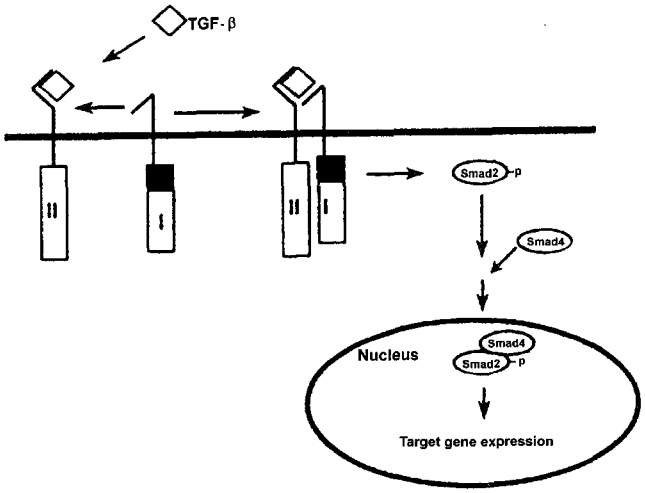

Fig. 1. The TGF-β signaling pathway

the target gene (Chen *et al.*, 1997). Inhibitory SMADs, induced by TGF-β family ligands, function in a negative feedback loop to terminate or reduce the strength

TGF-β regulates cell proliferation: Perhaps the most prominent activity of TGF-β is its ability to arrest the cell cycle and consequently inhibit cell proliferation (Alexandrow and Moses 1995). This effect is most profoundly observed in cells of epithelial type such as the mink lung epithelial cells, MvLu1A, mouse keratinocytes, and human colon epithelial cells. Addition of TGF-β to epithelial cell systems has been shown to produce myriad effects on the expression and activation of various cyclins and cyclin-dependent kinases (cdk's). During early G1 phase, addition of TGF-β markedly inhibits induction of CDK4 thus blocking entry into S phase (Ewen *et al.*, 1993). In HaCaT cells but not Mv 1Lu cells, TGF-β1 is capable of completely shutting down induction of cyclin E and CDK2 later in G1 phase. TGF-β also inhibits induction of cyclin A during late G1 phase. There is indirect evidence that TGF-β may regulate the activity of p27KIP1, a postulated inhibitor of the cyclin-E-CDK2 complex (Datto *et al.*, 1995). TGF-β has also been found to increase expression of p15INK4B and p21, another CDK inhibitors whose precise role in the cyclin pathway are currently under investigation (Li *et al.*, 1994). Progression through the cell cycle depends on phosphorylation of the Rb protein which is also inhibited by TGF-β (Ewen *et al.*, 1993).

Development of TGF-β resistance during malignant progression: The possibility that defects in the TGF-β receptor system might contribute to the development of resistance to the growth inhibitory effects of TGF-β was first introduced by Sporn and Roberts over ten years ago (Kim and Kim 1996; Roberts and Sporn, 1990).

Prior to cloning of the TGF-β receptors, it had been observed that retinoblastoma cells were resistant to inhibition of growth by TGF-β and did not demonstrate phenotypic expression of TGF-β receptors (Kimchi *et al.*, 1988). Similarly, PC12 cells derived from rat pheochromocytoma were TGF-β resistant and lacked demonstrable levels of TGF-β receptors.

Kadin *et al.* (Kadin *et al.*, 1994) reported a study involving cutaneous T-cell lymphomas, a group of tumors notorious for slow progression following a period of spontaneous regression. While a cell line derived from an early tumor was growth inhibited by TGF-β, two separate cell lines derived from the same tumor at a later, more progressive stage demonstrated no such inhibition of growth (Kadin *et al.*, 1994). The cell line from the early tumor was found to possess a normal complement of TGF-β type I and type II receptors, while neither type I nor type II receptors were detectable by crosslinking in the unresponsive cell lines. Identical T-cell receptor alpha-chain rearrangement and a common balanced translocation confirmed that all cell lines were derived from the same clonal cell population. The same investigators also examined malignant T-cell lines from Sezary syndrome patients and one noncutaneous T-cell lymphoma and found similar loss of TGF-β receptors (Capocasale *et al.*, 1995). Northern analysis of these cell lines revealed clearly detectable levels of mRNA for the type II receptor, and the authors proposed that this finding suggested a defect at the protein level.

Following this report, numerous other malignancies have been examined and individual tumors have been found to have developed resistance to growth inhibition by TGF-β (Filmus *et al.*, 1992; Manning *et al.*, 1992; Park, *et al.*, 1994; Sun *et al.*, 1994). These tumors also possess decreased or absent levels of receptors for TGF-β and include such diverse malignancies as gastric cancer, colon cancer, small cell lung cancer, hepatoma, squamous cell carcinoma, breast cancer, endometrial carcinoma, bladder cancer, and osteosarcoma.

TGF-β receptor mutations in human malignancy

The phenomenon of acquired resistance to growth inhibition by TGF-β as witnessed in various tumor cell lines has been accepted for some time. However, the first direct evidence that resistance to TGF-β can develop through the acquisition of a mutation in a receptor gene came in 1994 with the discovery that a majority of gastric cancer cell lines studied were resistant to the growth inhibitory effects of TGF-β and that this resistance correlated with gross structural defects of the type II receptor gene (Chang *et al.*, 1997; Park *et al.*, 1994). There was no correlation with abnormalities in the type I or type III receptor genes. A total of eight different tumor cell lines were examined by Southern and Northern analysis. Two of the cell lines, SNU-5 and SNU-668, demonstrated deletion of the the type II receptor gene after exon 2 in SNU-5 and after exon 3 in SNU-668 and, as expected, produced truncated TGF-β type II receptor transcripts. Two cell lines, SNU-601 and SNU-719 showed amplification of the type II receptor gene and corresponding amplification of type II receptor mRNA. Two other cell lines, SNU-1 and SNU-638, produced normal appearing bands on Southern blot but undetectable levels of mRNA. The only cell line that was growth inhibited by TGF-β, SNU-16, was also the only cell line to possess a normal type II receptor gene by Southern analysis and the sole cell line to produce normal levels of mRNA.

Mutation of the p53 gene, considered a relatively late step in the carcinogenesis pathway, was also identified in four of six gastric cancer cell lines examined (Park and Kim 1994). One of these cell lines, SNU-16, expresses normal type II receptors and is growth inhibited by TGF-β which suggests that mutation of

the TGF-β RII gene may occur even later in the course of malignant progression or may be required for metastatic behavior. Numerous additional mutations have been associated with gastric carcinoma, including point mutation of the ras oncogene, amplification of c-*met*, K-*sam* and c-*erbB*-2/*neu* as well as loss of heterozygosity at bcl-2, APC, and DCC gene loci. However, the frequency of the TGF-β RII mutation appears to be as high or higher than any other single mutation reported in human gastric carcinomas. Escape from TGF-β mediated growth inhibition may therefore be a threshhold achieving event determining a tumor's evolution towards malignancy.

A major breakthrough in understanding mutagenesis of the TGF-β type II receptor occurred with a recent report involving a specific subpopulation of human colon cancers, termed hereditary nonpolyposis colon cancer (HNPP). A characteristic feature of these tumors is a defect in DNA replication error repair leading to the phenomenon of microsatellite instability and what has been termed the RER+ (replication error positive) phenotype (Eshleman and Markowitz, 1995). Defective DNA repair in these tumors is highly correlated with mutations of the TGF-β type II receptor gene (Markowitz *et al.,* 1995; Myeroff *et al.,* 1995; Parsons *et al.,* 1995; Wang *et al.,* 1995). The characteristic finding is a frameshift mutation affecting a ten nucleotide poly-adenine repeat region adjacent to the transmembrane domain of the gene. Insertion or deletion of one or two adenines between nucleotides 709 and 718 shift the reading frame and lead to premature chain termination from newly created downstream stop codons. The resultant truncated type II receptor proteins are 129 to 161 amino acids in length in contrast to the wild type length of 565 amino acids and lack the activating serine/threonine kinase domain. Affected cell lines demonstrate no detectable surface receptors by crosslinking assays and are not growth inhibited by TGF-β. To confirm that this mutation was not a cell culture artifact, DNA from the original tumor specimens for three cell lines was examined and found to harbor identical mutations. Normal tissue adjacent to tumor within the same specimens did not contain the mutation.

Transcriptional regulation of the type II receptor

In a previous study, our laboratory described a series of gastric cancer cell lines in which resistance to TGF-β correlated with gross structural mutations in the type II receptor gene (Park *et al.,* 1994). There were, however, two notable exceptions in which Southern analysis yielded a gene without gross deletions or rearrangements, yet no type II receptor protein or mRNA was produced (Inagaki *et al.,* 1993). This suggested that abnormalities in transcriptional regulation of the type II receptor might be involved in some instances of escape from TGF-β mediated growth inhibition.

The promoter region for the TGF-β type II receptor gene has been cloned and sequenced (Bet *et al.,* 1995). Several positive and negative transcriptional regulatory elements have been identified and positioned, and the relevant target sequences for three putative novel transcription factor complexes have been reported. Basal levels of transcription are determined by the core promoter element in cooperation with both PRE1 and PRE2 (positive regulatory elements 1 and 2). 70 percent of core promoter activity is directed by an Sp 1 site. PRE1 consists of nucleotides -219 to -172 relative to the published transcription start site and contains two discrete target sequences which bind an AP1/CREB-like transcription factor in addition to an unidentified novel transcription factor complex. PRE2 is located between +1 and +50 relative to the published transcription start site and contains two overlapping target sequences, both of which appear to bind novel transcription factor complexes. Given the importance of receptor expression levels, particularly of the ligand binding type II receptor, for determining responsiveness to TGF-β, it is quite likely that transcriptional regulation of the expression of the type II receptor will emerge as a key mechanism controlling cellular responsiveness to TGF-β. This hypothesis is supported by the existence of such an intricate TGF-β RII promoter region composed of multiple positive and negative regulatory elements which are recognized, for the most part, by previously unidentified transcription factor complexes. Given the surprising number of possibly novel transcription factor complexes which have been identified as associating with the type II receptor promoter, it is compelling to speculate that the absolutely vital roles played by the TGF-β receptor-ligand system have led to the evolution of a unique collection of proteins dedicated to the regulation of this system.

There is concrete evidence that variation in expression of transcription factor proteins can lead to alteration in TGF-β receptor levels and that these alterations correlate with differing levels of responsiveness to TGF-β. Electrophoretic mobility shift assays were performed with nuclear protein extracts from different human gastric carcinoma cell lines and ^{32}P-radiolabelled double-stranded oligonucleotide probes representing the PRE1 and PRE2 target sequences. A cell line with normal levels of type II receptor was compared with cell lines underexpressing and overexpressing type II receptor. Early results showed variations in binding of two novel transcription factor complexes to PRE2. Increased binding of one complex correlated with increased expression of the type II receptor whereas increased binding of the second complex correlated with decreased expression of the type II receptor. We have recently identified proteins that recognize and

bind to the PRE2. The isolation and sequencing of one clone named ERT (Choi *et al.*, 1998) revealed it to be a member of the ets family of transcription factor. Expression of ERT correlates well with expression of TGF-β type II receptor in gastric and colon cancer cell lines.

Type I receptor defects in human cancer

Most of the attention over the past year has focussed on the prevalence of TGF-β type II receptor gene mutations in cancer and their role in the development of resistance to TGF-β-mediated growth inhibition (Garrigue-Antar *et al.*, 1995). The type I receptor, however, plays just as essential a role in TGF-β signal transduction, and it should not be surprising that mutations in the type I receptor gene can lead to a similar TGF-β resistant phenotype.

Kim *et al.*, (1996) recently reported the first confirmed type I receptor gene mutation in a human malignancy. Their study examined three prostate cancer cell lines, two of which were growth inhibited by TGF-β-PC3 and DU145, and one which had developed resistance to TGF-β-LNCaP. Western analysis and RT-PCR revealed that while all three cell lines express type II receptor, type I receptor was only expressed by the responsive cell lines PC3 and DU145, not by the unresponsive cell line LNCaP. Although the gene itself was not sequenced, southern analysis revealed a definite structural alteration within the type I receptor gene. Transient transfection of LNCaP cells with type I receptor cDNA successfully restored sensitivity to TGF-β.

CONCLUSION

Given the vast array of physiological functions normally regulated by TGF-β, the complex network of related ligands and receptors mediating its effects should come as no surprise. Similarly, given the tightly regulated, multi-tiered and diverging signal transduction pathway one should anticipate that interruptions in the normal operation of this pathway may occur at multiple levels. Although these other defects do undoubtedly exist and have yet to be found, it is noteworthy that a single element of this complex pathway, the TGF-β type II receptor, has already emerged as uniquely susceptible to derangement during the natural course of cellular growth and differentiation.

Evolution and preservation of such phenomenally complex organisms as the human species is vitally dependent on high-fidelity DNA replication. The existence of complicated cellular machinery responsible for duplicating and proofreading DNA in the course of cell division has been recognized for some time. Defects in these DNA repair mechanisms have been associated with development of several different cancer-susceptibility syndromes, e.g. Li-Fraumeni and xeroderma pigmentosum. The identity of the genetic targets most commonly disrupted as a result of defective DNA repair mechanisms is only now coming to light. Repetitive sequences of DNA appear particularly prone to duplication error, and the existence of two such repetitive sequences within the TGF-β type II receptor gene renders it an exceptionally vulnerable target for mutation. One type of mutation occurs in a poly GC stretch of DNA within the kinase domain of the receptor and was the first high-frequency gene mutation to be associated with the RER+ phenotype in the hereditary nonpolyposis colon cancer syndrome. This mutation appears to block TGF-β signaling by disabling the kinase activity of the type II receptor. The second type of mutation occurs within a sequence of ten repeating adenines near the transmembrane domain of the type II receptor. The resultant frameshift leads to premature chain termination and expression of a truncated inactive receptor.

Normal expression of the TGF-β type II receptor is clearly necessary for TGF-β to exert its growth inhibitory effects. The characterization of high-frequency mutations in the type II receptor gene leading to unrestrained clonal growth in several different human cancers establishes TGF-β RII as an important tumor suppressor gene. Its identification should allow development of novel therapeutic approaches to cancer which seek to repair or replace the defective receptor. In addition, recent studies have begun to dissect the complicated apparatus regulating normal transcription of the type II receptor. At some point in the future, more sophisticated therapy may target one or more transcription factors dedicated to the type II receptor.

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