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Targeted tumor therapy with the TGF-β2 antisense compound AP 12009

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Abstract

TGF-β overexpression is a hallmark of various malignant tumors. This is due to the pivotal role of TGF-β as it regulates key mechanisms of tumor development, namely immunosuppression, metastasis, angiogenesis, and proliferation. We have developed a new immunotherapeutic approach for the treatment of malignant tumors based on the specific inhibition of TGF-β2 by the antisense oligodeoxynucleotide AP 12009. After providing preclinical proof of concept, we assessed safety and efficacy of AP 12009 in clinical phase I/II open-label dose escalation studies in high-grade glioma patients. Median survival time after recurrence exceeded the up to date literature data for chemotherapy. A phase I/II study in pancreatic carcinoma and malignant melanoma is currently ongoing. Our results implicate targeted TGF-β2 suppression as a promising therapeutic approach for malignant tumor therapy.

Keywords: TGF-β; High-grade gliomas; Pancreatic carcinoma; Malignant melanoma; Antisense oligodeoxynucleotides

1. Introduction

Selection of an appropriate target proves to be the first and crucial step for determining success or failure of a drug candidate. An optimal molecular cancer target plays a key role in cancer disease progression by promoting the vital functions of the tumor, thus making it difficult to be bypassed by the tumor. An ideal candidate fulfilling this prerequisite is the transforming growth factor beta (TGF-β). TGF-β is a multifunctional polypeptide cytokine playing various roles in cell functions, including morphogenesis, cell proliferation, migration and is a key regulator of the immune system [1]. Three TGF-β isoforms are expressed in mammalian tissues: TGF-β1, TGF-β2 and TGF-β3 [2,3]. During embryogenesis all three TGF-β isoforms are expressed. In normal adult tissues TGF-β1 is by far the predominant isoform, while expression of TGF-β2 and TGF-β3 is much more restricted. Although the majority of human tissues are able to express both TGF-β ligands and their associated receptors, tumor cells are known to be an important source of TGF-β production [3,4]. Secreted TGF-β binds to TGF-β receptors (TBR) and initiates a signaling cascade via cytoplasmic signaling mediators, the so-called Smads, into the nucleus where the Smad complex regulates target gene expression [2,5].

In malignant tumor cells the TGF-β Smad pathway is usually mutated, while other pathways are still functional (for review [4,6]). Nevertheless, a wide variety of tumors even increase TGF-β secretion compared to the corresponding normal tissue [6,7] and the degree of TGF-β overexpression correlates with malignant progression. Besides the traditional Smad pathway, a number of other intracellular signaling pathways including mitogen-activated protei nn kinase (MAPK), N-Ras, Ral guanine nucleotide

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exchange factors (Ral-GEF), phosphatidylinositol-3-kinase (PI3-K/Akt), c-Jun-NH(2)-terminal kinase (JNK), p38-P and protein phosphatase 2A (PP2A/p70^{S6K}) are described [5,8–10,103]. Activation of these alternative signaling cascades appears to enhance malignant progression, thus amplifying TGF-β tumor promoting effects.

Over the past few years the significance of TGF-β has become increasingly apparent as this cytokine obviously elicits two opposite mechanisms depending on the respective environment [7,10,11]. In normal cells of epithelial origin as well as in early well-differentiated tumor cells of epithelial origin TGF-β acts as a suppressor of proliferation [1,12]. In contrast, in cells of mesenchymal origin, e.g. fibroblasts, and during malignant progression of epithelial cells to undifferentiated invasive malignant tumors, tumor cells become irresponsive to the antiproliferative effects of TGF-β [13]. Moreover, in many cases tumor cells are even growth stimulated by TGF-β. This phenomenon has in part been elucidated recently [3,11,14–19]. This process is associated with the process of epithelial to mesenchymal transition (EMT) [20,21]. During tumorigenesis cells of epithelial origin often acquire a mesenchymal phenotype. Thus, these cells can increase their ability to invade the extracellular matrix, a prerequisite for invading normal tissue and for forming metastases of these carcinomas [22]. A number of pathomechanisms including genetic and biochemical changes have been identified explaining the reason for TGF-β losing its growth regulatory effect selectively on malignant cells. The main targets in this process appear to be TGF-β receptors, Smads, and target genes modulated by TGF-β [15,23,24]. The dual role of TGF-β in tumor progression has been described in both experimental and clinical observations [25]. The conversion from an autocrine inhibitor to a stimulator of cancer cell proliferation during tumor progression is contrasted by the paracrine suppressive effect on those cells involved in the antitumor immune response including cytotoxic T-cells, NK-cells as well as B-cells. Furthermore, the increased level of TGF-β also affects the surrounding cellular environment in a paracrine manner including the extracellular matrix and blood vessel formation [13,26]. This pleiotropic effect on the tumor cells themselves, the tumor environment and on the escape mechanism from immunosurveillance (for review [27]) makes TGF-β a crucial tumor target gene. Amongst several key mechanisms by which TGF-β promotes tumor progression escape from immunosurveillance may be the most important. TGF-β has been repeatedly described to be the most potent immunosuppressant known [15].

While the relationships between TGF-β and cancer progression are multifaceted and complex, and are still not fully understood to date, significant progress has been made over the last years. In the early 90s our group realized that blockade of the activity of TGF-β in tumor tissue represents a novel most promising therapeutical approach aiming at the reduction of tumor growth, invasion and metastasis, epithelial to mesenchymal transition (EMT), angiogenesis and reversal of the escape from immunosurveillance ([28,29], Fig. 1). There are a large number of human tumors with a documented overexpression of TGF-β, e.g. malignant glioma [23,29], pancreatic carcinoma [30,31], malignant melanoma [32,33], prostate cancer [34–36] and various other malignant tumors (Table 1). The degree of TGF-β production by tumor cells has been shown to be correlated with aggressiveness and grade/stage of malignancy [7,10,15,27,29,37,38]. Targeting of TGF-β is currently tested in various ways including TGF-β binding molecules as, e.g. monoclonal antibodies and TGF-β receptor kinases ectodomains [39–41].[1] While these approaches can be promising they would not tackle the proposed intracellular effects of TGF-β [42,43]. Preventing the translation of this protein therefore may represent a significant advantage over inhibition of just its extracellular form. This is where antisense drugs come into account. Upon binding to the target, i.e. mRNA of specific genes, antisense drugs can block specifically the process of translation. As a consequence, the protein is downregulated or produced incompletely and thus remains inactive. The first antisense compounds so far have had limited effect, most likely due to the choice of both, suboptimal targets and insufficient selection of sequence (see Section 3.2).

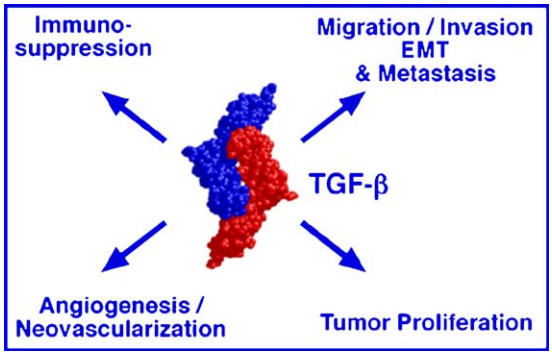

Fig. 1. Transforming growth factor beta (TGF-β) is a key regulator of cancer progression.

Table 1
Selection of tumors overexpressing transforming growth factor beta (TGF-β)

TGF-β overexpressing tumors	Reference
Malignant glioma	[23,29,90]
Pancreas carcinoma	[30,31]
Colorectal carcinoma	[91–93]
Non-small cell lung cancer (NSCLC)	[94]
Prostate carcinoma	[34–36]
Melanoma	[32,33]
Hepatocellular carcinoma	[95]
Hematological malignancies	[96,97]

[1] Although these approaches already showed promising results [40], in this review particular emphasis will be placed on the antisense technology.