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Review

From wavy hair to naked proteins: The role of transforming growth factor alpha in health and disease

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ABSTRACT

Since its discovery in 1978 and cloning in 1984, transforming growth factor-alpha (TGF-α, TGFA) has been one of the most extensively studied EGF receptor (EGFR) ligands. In this review, we provide a historical perspective on TGFA-related studies, highlighting what we consider important advances related to its function in normal and disease states.

Contents

1. Introduction

Transforming growth factor-alpha (TGF-α, TGFA) was the second member of the EGF receptor (EGFR) ligand family to be identified after the discovery of the prototypic member, EGF. TGFA activity was first discovered when researchers observed that sarcoma virus-transformed fibroblasts released a peptide growth factor activity that could block binding of EGF to cells, suggesting another growth factor competed with EGF for binding to cell surface EGFRs [1]. Later, this activity was partially purified from these transformed cells and induced growth in soft agar, the best *in vitro* correlate of tumorigenesis [2]. All three major peaks of activity (25, 12, and 7 kDa) competed with EGF for EGFR binding. This partially purified material was called "sarcoma growth factor" or

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simply "transforming growth factor". The discovery of transforming growth factors led to the concept of "autocrine signaling", that is, ligand is synthesized and released by a cell and then taken up by receptors on that same cell [3]. This novel concept helped explain growth factor independence, one of the defining properties of cancer cells: through autocrine stimulation, cancer cells are able to escape external growth controls [4].

A few years later, it was shown that the transforming growth factor activity consisted of two distinct peptide growth factors. The first bound to EGFR and was independently transforming when added to non-transformed fibroblasts; this was designated TGF-α. The second peptide, separated by HPLC, did not bind to EGFR and was not transforming on its own, instead it enhanced TGFA- or EGF-induced transformation of non-transformed fibroblasts [5]. This second peptide was named transforming growth factor-beta (TGF-β, TGFB1). Moreover, when added to epithelial cells, the two peptides exhibited distinct actions: TGFA stimulated growth, whereas TGFB1 was a potent growth inhibitor.

Rik Derynck cloned human *TGFA* in 1984 [6]. He showed it was an EGF homolog and bound to EGFR [6]. The *Tgfa* knockout phenotype resembled that of the *Egfr* knockout. In fact, the characteristic "waved" coat phenotype observed in *Tgfa* knockout mice had been reported much earlier (*waved-1, wa1*) [7]. In complementation studies, the groups of David Lee and Ashley Dunn showed that *wa1* mapped to the *Tgfa* locus [8,9]. *wa1* mice show reduced Tgfa expression, but the precise mutation has not been identified. *waved-2* (*wa2*) mice also show a similar coat phenotype; these mice have a mutation in the *Egfr* tyrosine kinase domain [10,11].

We, and others, have previously reviewed TGFA in the context of EGFR and its cognate ligands as relates to normal physiology and selected disease states [12–19]. Here we review what we consider to be important advances in the study of TGFA. We highlight our work that led to the isolation of Naked2 (NKD2), a novel trafficking adaptor for TGFA that also acts as a negative regulator of WNT signaling. We also discuss the role for TGFA in the pathogenesis of a rare premalignant hyperplastic disorder of the stomach, Ménétrier's disease.

2. TGFA: gene and protein structure

Human *TGFA* is located on the short arm of chromosome 2 (2p13) spanning a 138.7 kb region [6]. The 4326 base *TGFA* transcript encodes a 160 amino acid peptide. A shorter transcript using an alternative in-frame splice site that removes three bases has been reported that encodes a 159 amino acid peptide lacking Val 32. Characteristic of the EGF ligand family, *TGFA* mRNA is spliced from six exons; all five introns are spliced out adhering to the consensus GT–AG boundary rule (Fig. 1) [20]. In contrast, *EGF* is encoded by 24 exons, which encode nine EGF domains in its extracellular region. All the other ligands code for a single EGF domain. *EREG* and *EPGN* are encoded by five exons each, where the fifth exon also codes the 3′-UTR. The sixth *TGFA* exon codes for a large 3.5 kb 3′-UTR that shares a high degree of sequence identity with mouse *Tgfa*, suggesting a conserved regulatory role [20]. Incidentally, exon 6 encodes only the two terminal valines; alternative splicing involving intron 5 and exon 6 removes a large proximal region of exon 6 resulting in both the terminal valines replaced with four or five amino acids [21]. These splice variants that lack large regions of 3′-UTR regulatory sequences and terminal valines are observed in human keratinocytes, as well as cancer cell lines, and seem to be more efficient at imparting growth factor independence to CHO cells than longer TGFA transcripts [21].

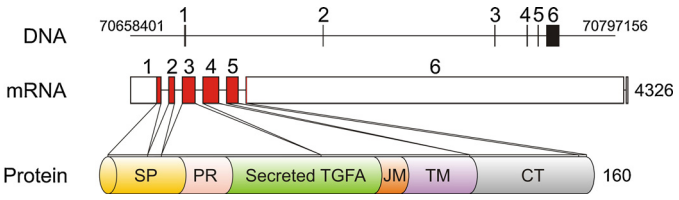

Fig. 1. TGFA gene, mRNA, and protein structure. Human *TGFA* is located on 2p13 and spans a 138.7 kb region. *TGFA* exons are dispersed unevenly in the genetic locus; these are numbered and indicated by vertical marks. *TGFA* mRNA is 4326 bases long; all six exons code for part of the final protein sequence; coding region is indicated in red. The first 248 bases in exon 1 comprise the 5′-UTR. Exon 6 at 3.5 kb is the longest, but only the first eight bases are part of *TGFA* coding region, resulting in a large 3′-UTR that ends in a 12 base polyadenylation tail. TGFA protein is synthesized as a 160 amino acid precursor. A 23 amino acid long signal peptide (SP) is followed by a 16 amino acid pro-region (PR). The secreted EGF-like domain of TGFA (highlighted in green) is 50 amino acids, which is followed by a nine amino acid juxtamembrane region (JM). The transmembrane domain (TM) and cytoplasmic domain (CT) are 23 and 39 amino acids long, respectively.

The 160 amino acid pre-proTGFA begins with a signal sequence of 23 amino acids at the amino terminus that is removed co-translationally during translocation to the ER lumen [22]. ProTGFA undergoes two metalloprotease cleavages at the distal and proximal sites to the EGF domain, enabling release of the mature soluble peptide into the medium that then binds and activates EGFR. The 23 amino acid transmembrane domain is followed by a 39 amino acid cytoplasmic domain.

Several post-translational modifications have been reported in TGFA. Characteristic of the EGF domain, three disulfide bonds are formed within this region of TGFA that involve six conserved cysteine residues. TGFA is also N-glycosylated in its extracellular domain; N25 is the only potential N-glycosylation site that has the conserved signature of a glycosylation sequon (NxS/T) [23,24]. TGFA O-glycosylation has not been reported. Cysteines at position 153 and 154 in the cytoplasmic domain are palmitoylated and this enhances the membrane association of TGFA [25]. Several detailed radiolabeling and pulse-chase experiments using different tagged and untagged TGFA constructs in various cell lines have shown consensus biosynthesis and processing of TGFA [17,26,27]. Nascent TGFA is synthesized as a 17–18 kDa peptide that is rapidly converted into a glycosylated 30 kDa form during transit from ER to Golgi. Within 30 min of appearance of the glycosylated cell surface form, cleavage proximal to the EGF domain leads to removal of the glycosylated region, resulting in a 17–18 kDa transmembrane form. This is followed by a second much slower (1–4 h) cleavage, distal to the EGF domain that gives rise to two fragments: a 5.6 kDa soluble TGFA and a 13–15 kDa membrane-anchored remnant that contains juxtamembrane, transmembrane, and cytoplasmic domains.

3. TGFA in physiological processes

Much has been learned about the role of TGFA in normal and disease states from mouse models in which *TGFA/Tgfa* has been overexpressed or knocked out. These studies combined with other mechanistic studies show that TGFA is involved in a number of cellular signaling pathways. Some of these actions are summarized in Table 1 showing different stimuli affect TGFA levels, localization, and activity, indicating TGFA is an important integrator of cellular signaling and function. A common action of TGFA is increased proliferation through activation of EGFR downstream signaling, but it may also mediate other functions like mucous production and inhibition of gastric acid secretion as seen in the overexpression models that mirror Ménétrier's disease. Below we will discuss the phenotypes and information gathered from these studies.