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Versteeg, H.H.

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In-silico Tissue Factor analysis; a ‘bit-by-bit’ comparison

Henri H. Versteeg¹, Sander J. van Deventer², Dick J. Richel³, Maikel P. Peppelenbosch⁴

¹Laboratory for Experimental Internal Medicine, Academic Medical Center, Amsterdam, The Netherlands
²Department of Gastroenterology, Academic Medical Center, Amsterdam, The Netherlands
³Department of Medical Oncology, Academic Medical Center, Amsterdam, The Netherlands

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The interaction of tissue factor (TF) with factor VIIa (FVIIa) is of crucial importance for hemostasis. In addition, TF/FVIIa binding has important coagulation-independent functions, especially in embryonic angiogenesis, oncogenic angiogenesis, leukocyte reverse transmigration, inflammation and the progression of lethal *E. coli* sepsis (reviewed in 1). With the discovery of a variety of signal transduction events elicited in TF expressing cells upon addition of FVIIa there is now little doubt that TF/FVIIa complexation not only stimulates hemostasis but also alters cellular physiology of the TF expressing cell, although the underlying molecular mechanism is controversial (1).

After its cloning in 1987, it was reported that the 295 amino acid TF polypeptide chain did not bear significant homology with other proteins. *In silico* studies, however, highlighted the high degree of structural similarity of TF with the super family of interferon receptors (IFNR)-α/β and γ (2). This notion was confirmed by the crystal structure of TF and it is often assumed that TF is derived from an ancestral gene that also gave rise to the cytokine receptor family. Today, however, much more sequence information as to TF and cytokine class II receptors in a variety of species is available, allowing better studies as to the inter-relationship between TF and the other members of the cytokine class II family.

We searched the available genomic databases for homologues of human (*Homo Sapiens*) TF and compared the sequences found to human IFNR-γ and mouse (*Mus musculus*) IFNR-α/β. We recovered a mouse, rat (*Rattus norvegicus*), guinea pig (*Cavia porcellus*) and Cow (*Bos Taurus*) TF sequence, of which the corresponding proteins have been functionally characterized. In addition, two highly homologous fish sequences were found in trout (*Oncorhynchus mykiss*) and puffer fish (*Takifugu rubripes*). The respectively short and absent intracellular domains of these sequences strongly suggests that these are representatives of a fish family of TFs rather than fish homologues of the IFNRs. Nevertheless, it is striking to

**Figure 1:** Schematic representation of genetic divergence based on the extracellular (A), transmembrane (B) and intracellular (C) domains of TF, IFNR-γ1 and IFNR-γ2. The protein sequences are derived from GenBank accession numbers: AAH11029 (human), P30931 (bovine), P24055 (rabbit), AAF36523 (guinea pig), NP_034301 (mouse), P42533 (rat), CAC82787 (trout), AAF47763 (fruit fly), EAA12120 (malaria mosquito), NP_032364 (mouse IFNR-γ2) and AAK30623 (human IFNR-γ1). The TF sequence of puffer fish is available on: http://www.ncbi.nlm.nih.gov/blast/db/blast.cgi?db=genomes%2Ffugu_GS&nt=0&ntl=72FuguGenscan_30764&RID=1034160711-025179-32615&segs=0-236&seal=451408229C30FCFA20C900CC5692E984.
In-silico TF comparison

Figure 1
note that the similarity of these fish sequences to either the human IFNR-γ or the mouse IFNR-α/β is almost as good as the homology to the mammalian TF sequences. Hence, the last common genetic precursor for IFNR and TF genes would appear to have existed shortly before the divergence between the Crossopterygii (from which the land-dwelling vertebrates have descended) and the Actinopterygii (the vast majority of contemporary bone fish) somewhere in the Devon era (330-290 million years ago). Furthermore, no IFNR sequences were detected in fish and thus TF appears the most ancient member of cytokine class II receptor family from which the IFNRs are relatively novel branches.

Interestingly, two insect sequences, one in the fruit fly (Drosophila melanogaster) and one in the malaria mosquito (Anopheles gambiae) and quite homologous to each other emerged from our search. The sequence homology of the extracellular domain of these sequences to the extracellular domain of vertebrate TF and IFNR indicates that these insect genes share a common ancestral gene with vertebrate cytokine class II receptors. No functional data are available with respect to these insect genes, but the resemblance of haemolymph coagulation to mammalian coagulation makes it tempting to suggest that these proteins are representative of an insect family active in lymphostasis. Less stringent homology searches revealed lists of extracellular prokaryotic proteins, most often involved in secretion system II. Thus, the cytokine class II receptor family has probably evolved from an ancient gene coding for a pre-eukaryotic extracellular protein.

We investigated the intracellular domain as the functional significance of this domain is under debate. The intracellular domain of TF is dispensable for many aspects of TF signal transduction, as evident by experiments in which intracellular-truncated TF is transfected baby hamster kidney cells (3). Moreover, mice genetically modified only to express a TF containing the extracellular and transmembrane regions did not display a phenotype (4). In contrast, the intracellular domain of human TF can directly interact with cytoskeleton via ABP-280 (5). This interaction is dependent on TF/FVIIa interaction and experiments using chimaeric molecules, containing the intracellular domain of TF and an unrelated extracellular domain, support a role for this interaction in mediating TF effects on cell spreading. Interestingly, we observed strong conservation between guinea pig, rabbit, cow, and human intracellular TF, but the homology of the intracellular domain of these species with mouse and rat TF was weak. Thus, evolutionary pressure on the sequence of the intracellular domain in the former species may differ considerably from that observed in the latter. This may
explain why murine genetics have proven unsuccessful in establishing a role for the intracellular domain.
References


