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Tumor Differentiation Factor (TDF) and its Receptor (TDF-R): Is TDF-R an Inducible Complex with Multiple Docking Sites?

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Abstract

Tumor Differentiation Factor (TDF) is a protein produced by the pituitary and secreted into the blood stream. TDF targets breast and prostate and induces cell differentiation. However, the mechanism of cell differentiation, the TDF receptor and the TDF pathway have not been adequately investigated. Here, we provide some insights about the possible composition of the TDF-R. TDF-R may be a protein complex, composed of GRP78, HSP70 and HSP90 proteins, and all three protein subunits have a docking site for TDF-P1. The question of whether the TDF-R complex is a stable or transient/inducible complex is currently being investigated.

Keywords: Tumor differentiation factor; Protein complex; Protein-protein interactions

Introduction

Virtually, all expressed proteins in a given cell are arranged into multi-protein complexes [1-9]. Identification of individual components of those complexes is extremely important for their functional characterization [5,8,10-20]. One of the most powerful methods in identifying proteins is mass spectrometry, in particular, Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) [5,8,15-19]. Combination of LC-MS/MS with a biochemical purification or fractionation strategy makes LC-MS/MS even more powerful, as the protein fractionation allows the LC-MS/MS to increase the number of proteins identified from a particular sample. Affinity Purification-Mass Spectrometry (AP-MS), a combination of Affinity Purification (AP) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), allows for screening of multiple-protein complexes, and for accurate identification of their components [6,7,9,13,14,21]. Therefore, a large number of the available Protein-Protein Interactions (PPIs) data, both stable and transient, have been discovered using AP-MS [2,22-26]. Using one protein as bait in AP-MS experiments will usually lead to identification of several potential interactors, and will help to organize them into functional interacting units. Tumor Differentiation Factor (TDF) is a protein produced by the pituitary and secreted into the blood stream. TDF targets breast and prostate and induces cell differentiation. However, the mechanism of cell differentiation, TDF receptor and TDF pathway, have not been thoroughly enough investigated. Here, we provide some insights about the possible composition of the TDF-R, as well as a review of research to date.

Methods

All biochemical purification and proteomics identification of the TDF-R candidates were performed, as described in [22,23]. All STRING PPIs were performed as in [4,27-31]. All structural biology experiments were performed as in [3,22,23].

Results and Discussion

Tumor Differentiation Factor (TDF) is a protein produced by the pituitary and secreted into the blood stream [32-35]. The target organs as breast and prostate, and the final effect is cell differentiation [32-34]. Work in our lab also identified TDF in the brain, specifically in neurons, but not in the astrocytes. Additional work in our lab also focuses on identification of the mechanism of TDF-induced cell differentiation. Therefore, some of the questions that we initially asked were 1) what are the potential TDF receptor (TDF-R) candidates? 2) How does TDF-R transduce the differentiation effect across the cell membrane, 3) Is TDF a hormone? To answer to one of these questions, we used TDF-P1, a 20 amino acid peptide from the open reading frame of TDF protein, cross-linked to agarose beads to purify potential TDF-R candidates. In our experiments using DU145 prostate cancer cells and MCF7 breast cancer cells, but not in experiments using HeLa, fibroblasts or other cells, we identified several proteins from the 70 kDa and 90 kDa family of Heat Shock Proteins (HSPs) as TDF-R candidates, with glucose-regulated protein/HSPA5/GRP78, HSP70 and HSP90 being the most likely TDF-R candidates [7,22,23,34,36]. Examples of MS/MS spectra that led to the identification of these proteins as TDF-R candidates are shown in Figure 1. The results from our AP-MS experiments could potentially expand the interactome map for those proteins and lead to better understanding of their function in breast and prostate cancer.

To further investigate GRP78, HSP70 and HSP90 proteins as potential TDF-R candidates, and whether these proteins interact with each other and possibly form a protein complex, we have used String database to predict Protein-Protein Interactions (PPIs) and the protein's functional relationships with its partner proteins [28,29,31]. We took dnaK (chaperone HSP70, co-chaperone with DnaJ; *Escherichia coli* strain K-12 substr. MG1655), 78 kDa glucose-regulated protein (heat shock 70kDa protein 5 or HSPA5) and HSP90 (heat shock protein 90kDa alpha) as examples to study their interaction and relation to their functional partners. Network architecture of protein-protein interactions and their functional relatives can be identified and estimated using String. String network (direct and indirect relations) uses several active prediction methods that include "co-expression", "experiments" and "text mining". (Figures 2A and 2B, 3A and 3B, and 4A and 4B) display possible network of multiple interacting partner proteins (nodes) of dnaK, HSPA5 and HSP90, respectively. A node is the representative of a protein and an edge is the interaction or linkage between two protein partners. Figures 2C, 3C and 4C are the graphic representation of the observed connectivity between dnaK/GRP78/HSP90 protein and their ten predicted partners. All these views are in

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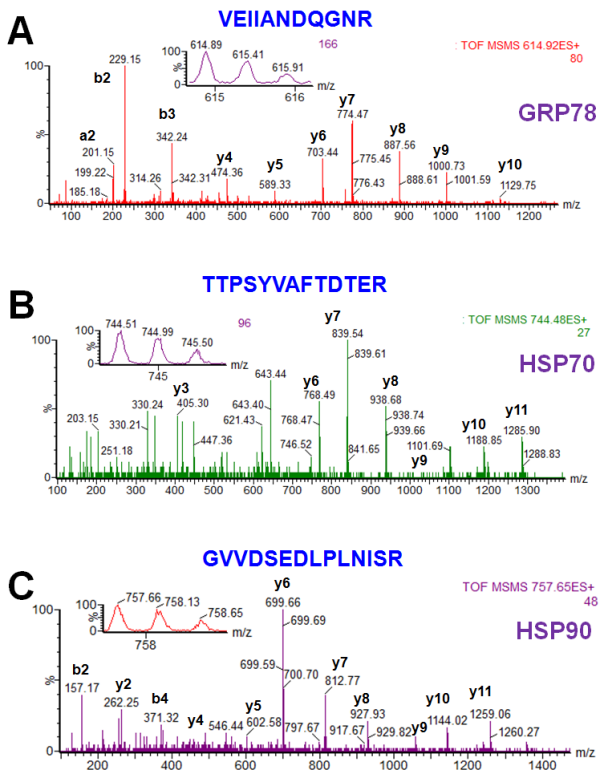

Figure 1: Identification of TDF-R candidates in DU145 cells using AP and LC-MS/MS (AP-MS). The potential receptors for TDF protein were purified from cell lysate using AP, resulting samples were separated by SDS-PAGE and the gel bands were excised and digested by trypsin. The peptides mixture was analyzed by LC-MS/MS to identify the purified proteins. A: MS/MS spectrum of peptide VEIIANDQGNR that led to identification of GRP78 as TDF-R candidate. B: MS/MS spectrum of peptide TTPSYVAFTDTER that led to identification of HSP70 as TDF-R candidate. C: MS/MS spectrum of peptide GVVDSEDLPLNISR that led to identification of HSP90 as TDF-R candidate.

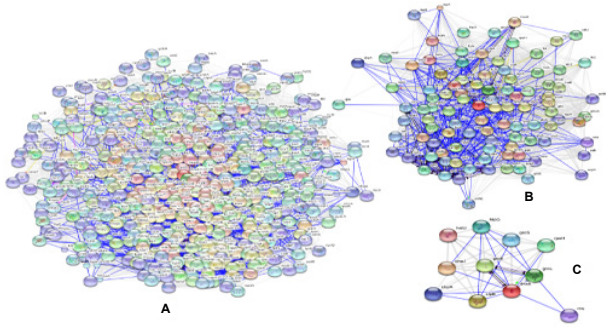

Figure 2: Model interaction network of dnaK chaperone (HSP70) and its possible functional partners. A and B displayed network of approximately five-hundred and one-hundred potential interacting partner proteins (nodes) of dnaK. C. Closer view of interaction. Here the numbers of interacting proteins are ten. These views are in confidence view, where denser lines describe stronger associations. These protein-protein interactions network was generated using STRING program, where a node represents a protein structure and links are projected by edge. The confidence score was set to 0.4.

action view, where dark lines describe stronger associations. Based on the published results and the String PPI network, it looks indeed as if these three proteins do interact with each other and possibly form a protein complex.

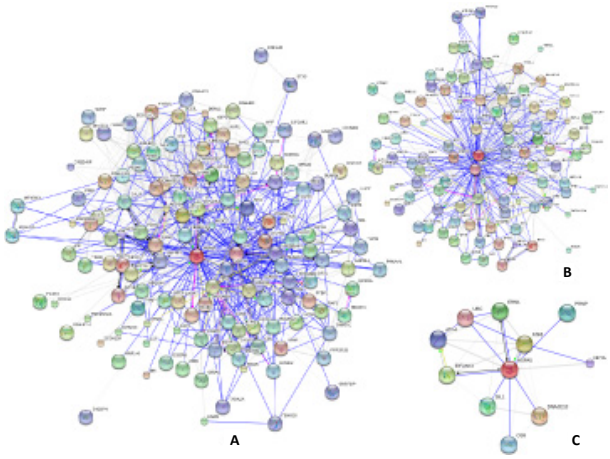

Figure 3: Model interaction network of 78 kDa glucose-regulated protein (GRP78/HSPA5) and its possible functional partners. A and B) Network of multiple potential interacting partner proteins (nodes) of HSPA5. C) Closer view of interaction. Here the numbers of interacting proteins are ten. The darker lines describe stronger associations. These protein-protein interactions network was generated using STRING program, where a node represents a protein structure and links are projected by edge. The confidence score was set to 0.4.

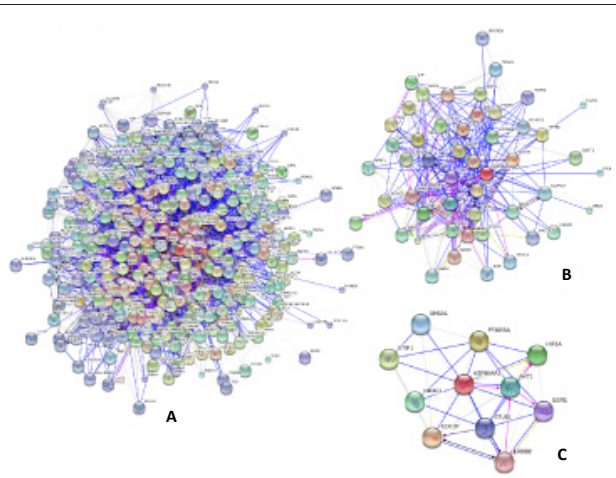

Figure 4: A model protein interaction network of HSP90 (heat shock protein 90kDa alpha) and its probable functional partners. A and B) Network of approximately five-hundred and one-hundred potential functional partner proteins (nodes) of HSP90. C) Closer view of interaction. Here the numbers of interacting proteins are ten. The thicker lines describe stronger associations. These protein-protein interactions network was generated using STRING program, where a node represents a protein structure and links are projected by edge. The confidence score was set to 0.4.

Treatment of MCF7 human breast cancer cells and DU145 prostate cancer cells with TDF-P1 leads to differentiation of these cells; this effect is not observed on other non-breast, non-prostate cancer or normal cells [32,33]. TDF-P1 is a peptide from the N-terminal part of the TDF that has demonstrated differentiation activity on breast and prostate cancer cells as the full length protein [32,33]. Therefore, to interact with TDF-P1 and transduce a differentiation signal, the three TDF-R candidates (GRP78, HSP70 and HSP90) must be present at the cell surface. However, it is still not clear to us whether these proteins form a stable protein complex or is a transient, inducible protein complex. This question is still being investigated in our laboratory. Also, not known is whether the knock down of GRP78, HSP70 and HSP90 will prevent binding of TDF and TDF-P1 to its receptor and will promote cell differentiation. This question is currently investigated in our laboratory.

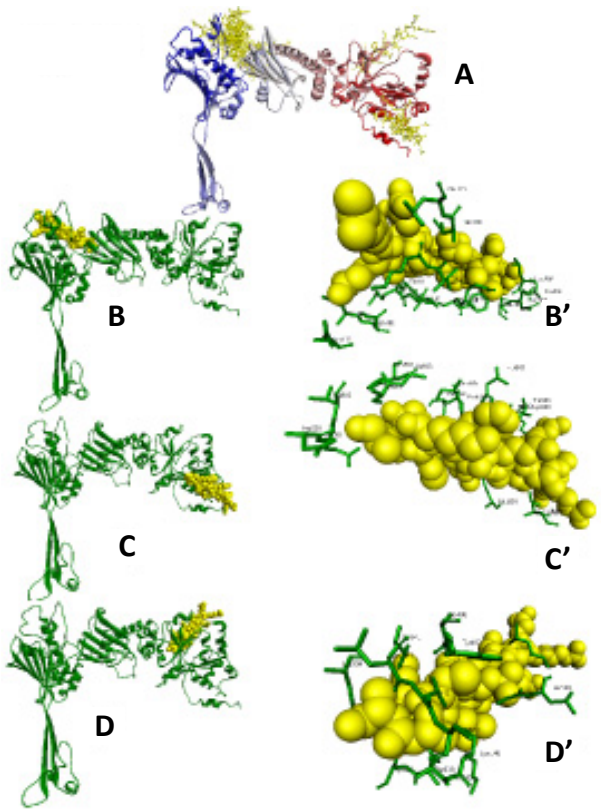

Figure 5: Possible docking sites of TDF-P1 on the model receptor protein as identified by "GRAMM-X". A: Predicted docking sites and poses of docked peptide (yellow). The secondary structure of the model receptor protein is colored from N (blue) to C (red) terminus. B, C and D: Predicted P1 binding sites, with the receptor protein displayed in green ribbon. The P1 peptide is shown in the yellow space filled mode. B'-D': Neighboring amino acid residues of docked P1 on the model receptor protein

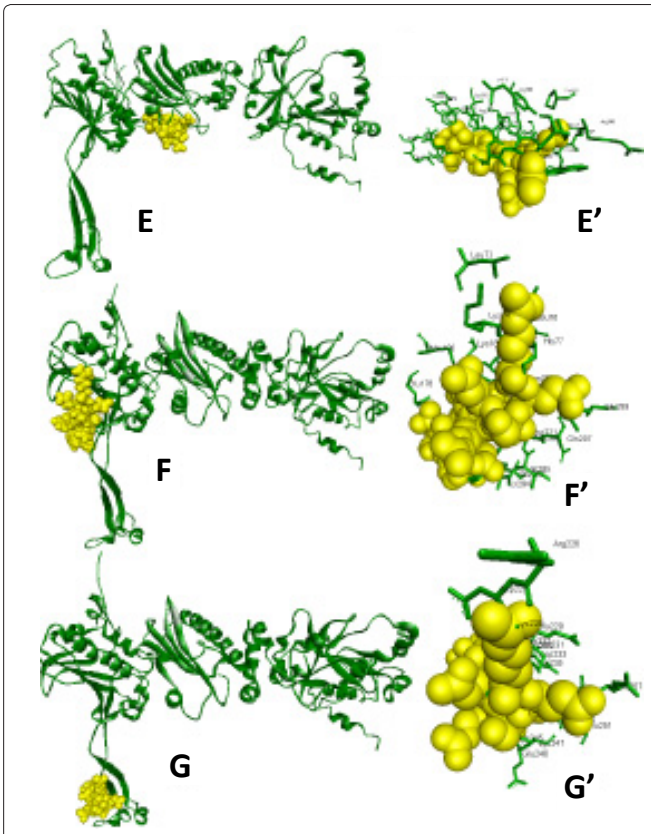

Figure 6: Three additional potential docking sites for TDF-P1, as predicted by using "Patch dock" and "Fire dock".

The next question that we asked was whether HSP90, in addition to GRP78 and HSP70, have docking sites for TDF-P1. We already knew that both GRP78 and HSP70 have several docking sites for TDF-P1. Therefore, we investigated HSP90 for possible TDF-P1 docking sites. The crystal structure 2CG9 (chain B, heat shock protein 90-alpha) was used as a template to set up a homology model of HSP 90 [37]. HSP 90 proteins are composed of N terminal, middle and C terminal domains. Figure 5A presents the homology model of 2CG9B starting from the N (colored blue) to C (colored red) terminals, and the model receptor was established using the SWISS-MODEL server [38,39]. The α-carbon Root-Mean-Square Deviation (RMSD) of 2CG9B crystal structure and homology model is 4.13 Å [40]. The docking site of P1 peptide onto the receptor model was identified using the GRAMM-X Protein-Protein Docking Web Server v.1.2.0, as used in our published work [22,23,35,36,41,42]. A second run for this identification was carried out using the "Patch dock" and "Fire dock" servers [43-46]. Detailed descriptions of these docking experiments are described in our previous papers [22,23,35,36], and Discovery Studio Visualizer 3.5 was used to plot the tentatively identified binding pockets [47].

We then used structural biology to investigate the possibility that the members of the HSP90 family of proteins are a docking place for TDF-P1. Among the first 10 highest ranked structures developed by "GRAMM-X" web server, P1 was docked onto three regions of the model receptor (Figure 5A). These three potential docking sites and neighboring amino acid residues of P1 peptide are shown in Figures 5B-5D. "Patch dock" and "Fire dock" simulation servers identified three additional potential docking sites for P1 peptide. These three additional potential docking sites and neighbor residues of docked peptide on the receptor model are shown in Figure 6. Therefore, based on these investigations, our molecular modeling experiments indeed found possible docking sites within HSP90 for TDF-P1.

Conclusions

Overall, the data allowed us to conclude that the TDF-R may indeed be a protein complex, composed of GRP78, HSP70 and HSP90 proteins, and all three protein subunits have a docking site for TDF-P1. The question of whether the TDF-R complex is a stable or transient/inducible complex is currently being investigated. Current investigations in our laboratory will also allow us to clarify whether there is only one subunit as the main TDF-R, or there is more than one natural docking site for TDF.

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