Tumor Necrosis Factor Receptor Associated Protein 1: An Introduction

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Heat shock proteins (Hsps)

The Italian geneticist, Ritossa (1962), made a scrupulous observation of the change in the puffing pattern of the polytene chromosomes present in the salivary glands of *Drosophila busckii* with elevated temperature. This puffing of the chromosomes was subjected with limited parts of the chromosomes and thought to have cytoprotective roles (Ritossa, 1962) and is attributed to stress response. The stress response is therefore always associated with the transactivation of new set of genes, called as heat shock proteins (Hsps) (Lindquist and Craig, 1988). These proteins were initially termed as Hsps (Tissieres et al., 1974) since they were identified in response to heat stress, however Hsps can be induced by a diverse varieties of stimuli. Later it was found that Hsps are expressed in normal cells too and are highly conserved and ubiquitously expressed in all cells and tissuses (Hartl, 1996), and that their expression is induced in response to stress stimuli (Benjamin and Mcmillan, 1998) although they also perform essential functions under normal physiological conditions. The stress response is highly conserved between prokaryotes and eukaryotes (Koga et al., 1999).

The basic function of Hsps is found to be chaperoning other proteins, hence they are also termed as "molecular chaperones". Chaperones interact with other proteins to help in the maintenance of cellular protein homeostasis, especially helping native polypeptides to maintain in folding competent state, and refolding of denatured or damaged proteins to their native conformation under normal physiological conditions. Advanced/specialized functions of Hsps in subsequent years have identified that they even promote conformational stabilization and functional maturation of mutated gene products (Lindquist and Craig, 1988).

Due to subsequent discovery of several homologues and analogues of Hsps in the cytosol (Hartl, 1996) and intracellular milieu, for convenience, Hsps are classified into families based on their molecular weights. These are Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, Hsp10 and small Hsp families (Gething and Sambrook, 1992). If not all, some of the cytosolic Hsp analogues are present in mitochondria and are probably involved in performing similar functions.

Hsp90

In eukaryotic cytosol Hsp90 exists in two forms, Hsp90α (inducible form) and Hsp90β (constitutive form). Its homologs in the endoplasmic reticulum (ER) are called Grp94 and in the mitochondria are called Trap1 (Csermely et al., 1998). Since mitochondria are thought to have evolved from the bacteria, Trap1 must be similar to bacterial counterpart of Hsp90. The bacterial/prokaryotic Hsp90 is named HtpG (High Temperature Protein Gene) (Sato et al., 2010). Unlike Hsp90 in the eukaryotic system, HtpG expressions under normal physiological conditions are dispensable for survival (Bardwell and Craig, 1988). However, subsequent studies have indicated that HtpG play a role in cytoprotection under stress conditions, which needs further understanding (Hossain and Nakamoto, 2003).

Interestingly, both Hsp90 and HtpG exist as phospho-dimers and exhibit chaperoning activity *in vitro*. The monomeric form of Hsp90 constitutes three major domains, N-terminal ATP binding domain (N- domain), middle domain (M-domain), and C-terminal dimerization domain (C-domain) containing the MEEVD motif (a tetratricopeptide repeat (TPR)) that helps in facilitating the protein-

protein interactions. The N-terminal domain and the middle domain are joined by a highly charged hinge region, which is thought to be involved in client protein interaction (Morra et al., 2012). However, Trap1 lacks this charged domain (Felts et al., 2000). Like any other chaperone, the N-terminus of Hsp90 also contains ATPase activity that helps in substrate binding and release in the chaperone cycle (Prodromou et al., 2000).

TRAP1

Tumor Necrosis Factor Receptor-Associated Protein 1 (TRAP1), a member of Hsp90 family gained importance when it was found associated with the cytoplasmic domain of type I Tumor Necrosis Factor Receptor-1(TNFR1) in a yeast two-hybrid screening system (Song et al., 1995). In an independent study using the same model system, another protein was found to be translocated into the nucleus under heat shock where it was bound with the simian virus 40 T-antigen-binding domain of hypophosphorylated retinoblastoma using its unique LxCxE motif and was named Hsp75 (Chen et al., 1996). However, later sequence analysis showed that both proteins were identical.

TRAP1, due to the presence of mitochondrial localization signal at its N-terminal end is primarily a mitochondrial matrix residing protein (Felts et al., 2000). However, studies have shown that they can be present at specific extra-mitochondrial sites (Cechetto and Gupta, 2000). Although the functional aspect of non-mitochondrial localizations of TRAP1 is less characterized, a recent study has shown that TRAP1 interacts with TBP7 and regulates quality control of proteins destined to mitochondria (Amoroso et al., 2012).
TRAP1 has high sequence homology with members of Hsp90 protein family (Song et al., 1995) and forms a tight homodimer with significant increase in its ATPase activity upon heat shock condition. Due to this structural similarity with Hsp90, especially conserved in the N-terminal ATP binding region, TRAP1-ATPase activity can be inhibited by the Hsp90 inhibitors like geldanamycin and radicicol. In contrast, TRAP1 cannot bind with the Hsp90 co-chaperones and facilitate conformational maturation of progesterone receptor (Felts et al., 2000). Besides these, the TRAP1 structure also lacks the hinge region found in the Hsp90 (Song et al., 1995).

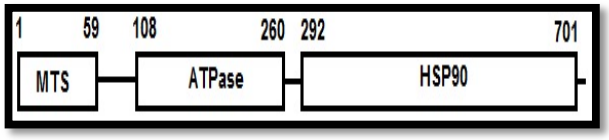

Figure 2.3 Domain organization of human TRAP1. The numbering refers to the amino acid sequence. MTS – Mitochondrial Targeting Sequence. [Adapted from Matassa et al., 2012].

TRAP1 and Cancer

TRAP1 has been shown to protect cancer cells from oxidative stress and apoptosis (Gesualdi et al., 2007). The TRAP1 level has been found to be higher in the mitochondria of breast, lung, colon, and pancreas and prostate cancer cells when compared to normal matched cells (Kang et al., 2007; Leav et al., 2010) indicating specialized functions. A microarray study suggested that TRAP1 can affect cell cycle too. Further, TRAP1 expression correlated with cell motility and metastatic spread (Liu et al., 2010).
Hence, TRAP1 may be regarded as the next potential candidate for therapeutic targeting in fight against cancer.

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