

Bioinformatics Analysis of HSP90 in Amniotic Fluid and Amniotic Membrane Cells as A Potential Anticancer Drug Target

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Abstract

HSP90AA is a crucial protective chaperon protein in normal and cancer cell. It has been previously determined HSP90AA down regulate in tumor cell treated with extract of amniotic membrane (AM) stem cells. Aim of this study find out the pathways and molecules associated with reduction of HSP90AA by bioinformatics methods. Network analysis defined the principal functions of HSP90AA in cancer survival and proliferation through interaction with important proteins with different functions such as AR, AKT, HIF1A and P53 to promote tumor cell proliferation and angiogenesis. Gene ontology analysis released the regulation of nitric oxide synthesis activity. In sum up results distinguished the misexpression of HSP90AA may due to down-regulation of its transcription factors or systemic changes in gene expression of cancer cell after treated with extract of AM cells and induce apoptosis in oxidation state.

Keywords: *Hsp90AA, Cancer, Amniotic membrane, Epithelialstem cells, Mesenchymalstem cells, Apoptosis*

Introduction

Amniotic membrane (AM) is an avascular stromal matrix that forms the innermost layer of the placenta. This tissue is composed of three main layers: an epithelial monolayer, an acellular basement membrane, a mesenchymal layer [1]. It has been widely used for clinical therapeutic applications. AM has unique properties such as anti-angiogenic, anti-inflammatory, anti-bacterial, anti-viral characteristics, as well as anti-scarring, and proapoptotic features [1, 2].

Meanwhile AM has low to moderate expression of the major histocompatibility complex (MHC) class I molecules human leukocyte antigen (HLA)-A, HLA-B, and HLA-C and express negligible levels of the MHC class II molecules HLA-DQ and HLA-DR [3]. Therefore it does not have problem of immunological transplant rejection.

Moreover AM is available tissue and its use has no ethical issue [3, 4] These attractive characteristics justifies the utilize of AM for vary clinical applications. Anti-tumor activities of AM were assumed first by Seo et al., they suggested that AM is suitable for cancer therapy because of its anti-angiogenic, pro-apoptotic and immune regulatory properties [5]. After that several studies confirmed the anti-cancer effects of the AM. In spite of many researches on anti-cancer properties of AM, the exact molecular mechanism of this effect is still unknown. Magatti et al demonstrated that yet-unknown soluble factors released from AM could inhibit cancer cell proliferation [6].

They reported that these factors arrested cancer cells in the G0/G1 phase of the cell cycle through modulating of key gene expression involved in cell cycle regulation [6, 7]. Authors observed down-regulation of

the expression of CDK4, cyclin D2, cyclin E and Cullin-1 that are involved in block of cell cycle progression [6].

Moreover other studies indicated up-regulation of negative regulators of cell cycle, particularly, p21 and p15 may be related to anticancer properties [6]. Niknejad et al explain a possible mechanism for the AM anti-cancer features focused on heat shock proteins (HSPs) in particular HSP90. HSP90, play a crucial role in development of tumor genesis. These researchers elucidated AM components exerts their anti-cancer effects including inhibition of angiogenesis, induction of apoptosis and cell cycle arrest through the inhibition of HSP90 [8]. In this study will be discussed HSP90 as a drug target for the AM anti-cancer effects by bioinformatics approaches and its role in protein-protein interaction network.

Materials and Methods:

All of the metabolic and non-metabolic pathways were obtained from the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database and the DAVID website (<http://david-d.ncifcrf.gov>) was used to perform KEGG pathway enrichment analysis. Use KEGG database to categorized HSP90AA Orthology which contain the pathways related the proteins [9] All proteins that interact with HSP90AA1 was found in STRING Database (version 10). This website (<http://string-db.org>) provides a critical assessment and integration of protein-protein interactions (PPIs), including structural and functional associations [10]. All proteins imported to cytoscape 3 as an open-source tool [11]. The PPI network was visualized by application of cytoscape software.

The important properties of network such as node, edges and hubs; the high degree nodes in network, are distinguished by this software. The plug-in unit of Cytoscape Cluster ONE [12], were used to perform network analysis and cluster analysis to find

the hub proteins of the network module. A Gene ontology (GO) category including biological processes (BP), cellular component (CC) and molecular function (MF) of proteins was used to identify the functions of network. Clue GO v2.0.5, Cyto scape plug-in tool, [13] visualizes and statistically evaluates genes/ proteins in a functionally grouped network.

Results

KEGG Orthology of HSP90AA have released in table 1. It contains the function of protein in normal and disease states. The integrated PPI network was obtained from STRING database by the application of Proteomics Standard Initiative Common Query Interface (PSICQUIC) source. The proteins that interact with HSP90AA were displayed in Fig.1. All of them contain CDC37, PTGES3, STIP1, HIF1A, AKT1, NR3C1, AHSA1, STUB1 and ESR1 were imported to cytoscape 3 software to visualize the Protein-Protein Interaction network (Fig. 2).

This network contains 74 nodes and 62 edges. Statistically network analysis was performed to determine different important parameters of network such as detected the nodes with the most degree are ESR1, AKT1, NR3C1 and HIF1A. It is important to mention that HSP90AA1 is a crucial node because is located between two HUBs. Fig.3 highlighted the HSP90 in network and their direct connections. Picture depicted that on the one hand ESR and STIP1 regulated the HSP90; on the other hand HIF1A is downstream of HSP90AA. In Fig. 4 also depicted analysis by Cluster ONE that finds overlapping protein complexes in a protein interaction network. Red nodes represent overlapping protein complexes which one of clusters contains HSP90AA1. Fig. 5 represented GO analysis of network by Clue GO- Clue Pedia. GO analysis determined the most important process related the regulation of nitric oxide synthesis activity and regulation of hormone metabolic process.

Table 1: KEGG Orthology of HSP90AA

Main categories	Sub categories	Pathway
Genetic Information Processing	Folding, sorting and degradation	Protein processing in endoplasmic reticulum
Environmental Information Processing	Signal transduction	PI3K-Akt signaling pathway
Organismal Systems	Immune system	NOD-like receptor signaling pathway

Human Diseases	Endocrine system	Antigen processing and presentation
		Estrogen signaling pathway
	cancer	Progesterone-mediated oocyte maturation
		Prostate cancer



Figure 1: PPI network of HSP90AA1 constructed by string database.

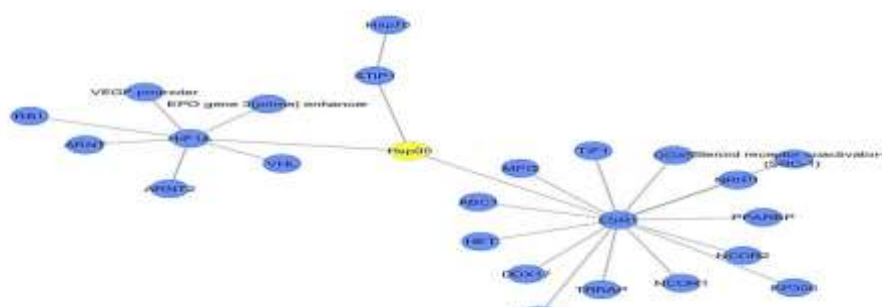


Figure 2: PPI Network of all identified proteins interacts with HSP90AA1 based on cytoscape 3 software. HSP90AA1 was highlighted by yellow color



Figure 3: HSP90AA1 in directed network reconstructed by BIND which determined direction of regulation.

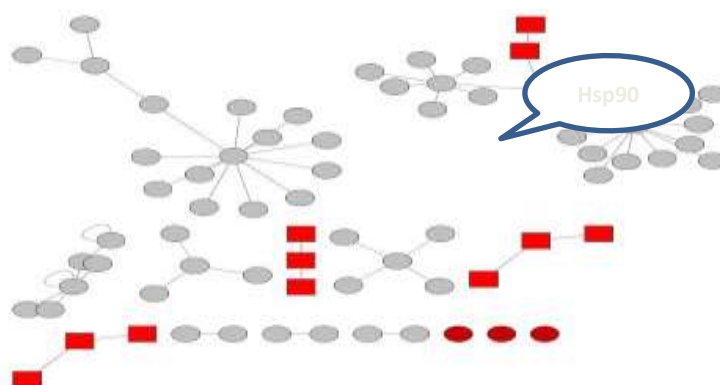


Figure 4: Red color represent overlapping protein complexes that identified by ClusterONE analysis

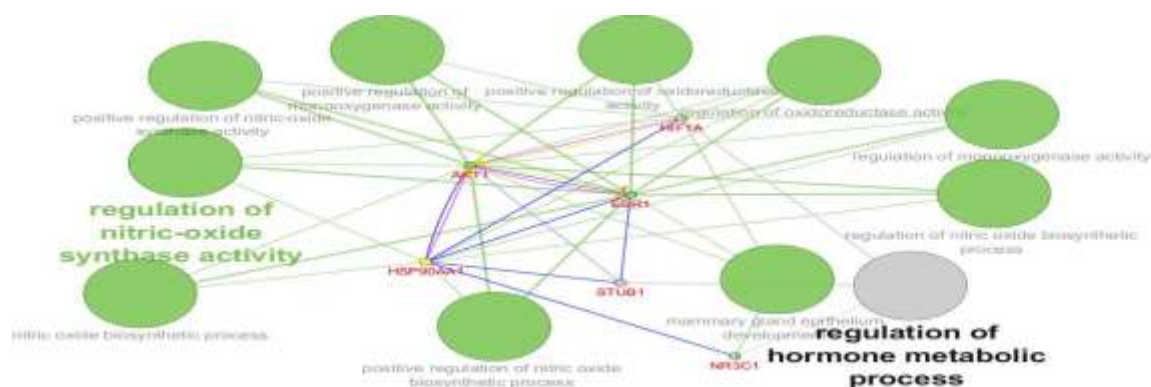


Figure 5: The GO analysis of network by ClueGO- CluePedia

Discussion

Hsp90AA as well as play several roles in normal cells such as a general protective chaperon (14, 15) interaction with 26s proteasome to degrade proteins, interaction with steroids receptors [16, 17], it has different roles in cancer cells to adapt cancer conditions. Generally overexpression in cancer cell has a protective effect to progress and survived cancer cells. As seen in kegg result (table1) imply that this protein play a crucial role in proliferation and survival cancer cells. Previously determined the HSP90 down regulated in cancer cell incubated with molecular extract of AM stem cells [6, 7]. Bioinformatics analysis of HSP90 demonstrate that several reason could be effect reduction expression of proteins including down-regulation upstream protein in cell signaling pathways, changing in interaction with its client proteins, down-regulation or disruption transcription factors function in protein complex of HSP90 gene expression, present of hsp90 inhibitor component in AM extract, disrupt function and finally degrade HSP90. According to table 1, hsp90 present in PI3K-Act signaling pathway in complex with different protein. The first molecules in this pathway related to growth factor receptors. When AM component block these receptors, cell don't need the production of hsp90 gene.

According to Fig.1, 2 and 3, HSP90AA1 interact with some important protein like CDC37 (microRNA), PTGES3 (disrupts receptor-mediated transcriptional activation), HIF1A (in embryonic

vascularization, and tumor angiogenesis), AKT1 (Mediates the antiapoptotic effects of IGF-I), AHS1 and STUB1 (activator of HSP90AA1), ESR1 (regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues) and tumor protein p53 (10). Network analysis determined HSP90AA1 is a party hub that involved in cancer pathways. Directed network released that ESR1 and STIP1 effect on HSP90AA1. On the other hand HSP90AA1 effect on HIF1A. Network analysis also determined the most important protein interact with hsp90 like ESR. ESR, client of hsp90, plays several roles in normal cells and in KEGG pathways detected in pathway in cancer and prostate cancer. It interact in the androgen receptor (AR) in cytosol or cell membrane to promote the cell to avoid the apoptosis, cell survive and proliferation. In AM maybe exist the AR ligand antagonist that can degrade or decrease expression of Hsp90 that is required for glucocorticoid receptor activity [18].

NOS have been determined in wide range of tumor cells [19]. This enzyme by synthesis NO has a dual property in cancer; it is known as a promoting cancer process and as a toxic molecule induced cancer cell to apoptosis process [20].

As seen in figure 5, NOS interact with AKT, Hsp90, and ESR, so NOS from different signaling pathways be activated and exert different function, any way it is important to mention the fact that NOS and their isomers can progress the cancer, so disrupt its triggers such as receptor (like ER), transcription factors and its clients proteins such as hsp90 lead to cancer treatment.

The inhibition of hsp90 or upstream activator or other TF can eliminate the protective effect on cancer cell, consequently the cell process into apoptosis, previously has been reported NOS is activated by CAM and strongly dependent on cytosolic calcium concentration. The inhibition of hsp90 has disruption effect on NOS free radical production consequently produce ROS followed by telomere erosion and finally apoptosis in cancer cells [20]. Hsp90 inhibitors, such as geldanamycin (GA), 17-allylamino-17-demethoxy-geldanamycin, and radicicol (Antibiotic), as anticancer agents block Hsp90 and interaction with other upstream and downstream proteins including N-ras, K-ras, HER-2, c-Raf-1, Akt, and mutant p53, so target cancer cell to apoptosis or cytostasis [21]. Previously determined the peptide and drug that inhibit the hsp90 or its clients for example HSP90 decoy peptides blocked eNOS interactions with hsp90, ultimately this disruption appropriate this peptide for

different target therapy in disease associated with HSP90 impairment [22]. Today, there are modern methods based on a variety of arrays and chips that can be used to identify and evaluate the factors involved in stem cells derived amnion membrane which responsible the apoptosis of treated cancer cell [23-26].

Conclusion

In sum up, bioinformatics study based on network realized HSP90AA1 is a crucial node that its inhibition can disrupt cell cycle, so it is necessary to determine the component in Amniotic membrane by high throughput techniques

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