Inhibition of HSP90 could be possible mechanism for anti-cancer property of amniotic membrane

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Abstract
Amniotic membrane (AM), the innermost layer of the fetal membranes, is considered as a suitable candidate for cancer therapy. In order to develop the AM as a new cancer therapeutic approach, it is essential to understand the molecular mechanism of the AM anti-cancer properties. Previous studies demonstrated that anti-proliferative effects of the AM on tumor cells were associated with induction of cell cycle arrest. Moreover, it has been shown that unknown substances in the AM induce apoptosis in cancer cells and inhibit angiogenesis in tumors. In contrast to the effects of the AM, heat shock proteins (HSPs), in particular HSP90, play a crucial role in development of tumorgenesis. HSP90 inhibits apoptosis in cancer cells and enhances angiogenesis and cell cycle progression. Based on the opposite effects of the amniotic membrane ingredients and HSP90, we hypothesized here that possible mechanism of the AM anti-cancer effects is through inhibition of HSP90.

Introduction
Amniotic membrane (AM), as the innermost layer of the fetal membranes, is a suitable biomaterial with a variety of clinical applications. The AM consists of five layers including epithelial layer, basement membrane, compact layer, fibroblast layer and spongy layer which is the nearest layer to chorion. Epithelial cells in epithelial layer and mesenchymal cells in fibroblast layer possess stem cells properties which make them promising sources in stem cell research and therapy.

The AM has several biological properties which make it a useful tissue in clinical situations, including anti-microbial, anti-fibrosis and anti-scarring characteristics as well as low immunogenicity and immunoregulation [1]. The AM has successfully been used in ophthalmological surgeries [2], human skin injuries and burns [3–5], nerve regeneration [6] and as surgical dressings for reconstruction of cavities in the human body as well as a scaffold for cultivation and delivery of different cell types into the body [7]. However, little is known about anti-cancer property of the AM.

Anti-cancer property of the AM was first hypothesized by Seo et al. [8]. They suggested that anti-angiogenic, immunoregulatory and pro-apoptotic activities of the AM could make it applicable in cancer therapy. The anti-cancer effects of the AM were then demonstrated by Parolini et al. [9], Jiao et al. [10] and Kang et al. [11]. Although research on anti-cancer properties of the AM is in progress, there are little information and data on the exact molecular mechanism of these effects of the AM.

To describe a possible mechanism for the AM anti-cancer property, we focused on heat shock proteins (HSPs). HSPs are ATP-dependent molecular chaperons which regulate protein folding and inhibit aggregation of misfolded proteins. HSPs family is classified according to their members molecular sizes, including Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small heat shock proteins [12]. These housekeeping proteins have a significant role in tumor growth and invasion. Facilitating the function of numerous oncoproteins by heat shock proteins has been reported in cancer cells [13,14]. Among HSPs, heat shock protein 90 (HSP90) plays important roles in key signalings of malignancies [15].

HSP90 function has provided an attractive target in cancer treatment. In vitro and in vivo inhibition of HSP90 has shown efficient anti-cancer effects in prostate, pancreas and lung neoplasms [16–18]. Moreover, many well tolerated drugs have been introduced for application in clinic [19]. Clinical trials on HSP90 inhibition have been shown effectiveness in advanced and refractory solid tumors [20–22] and bone marrow malignancies [23,24].

Supporting evidences of hypothesis
Our hypothesis in this article is based on the following evidences:
Evidence 1

Parolini et al. recently reported that anti-proliferative effect of the AM is associated with induction of cell cycle arrest in G0/G1 phase by unknown soluble factors. They have suggested that the AM cells can down-regulate expression of cancer cells genes associated with cell cycle progression such as cyclins (Cyclin D2, E1 and H) and cyclin-dependent kinases (CDK 2, 4 and 6) \([9]\). Hsp90 also regulates most or all phases of cell cycle, but in opposite manner. Cyclins B, D and E and cyclin dependent kinases including CDKs 1, 2, 4 and 6, as major regulators of cell cycle, are directly or indirectly up-regulated by Hsp90. Inhibition of CDK 2 and cyclin E through inhibition of HSP90 leads to cell cycle arrest in G1/S boundary \([25]\).

Evidence 2

Another presented mechanism for anti-cancer characteristics of the AM is induction of apoptosis in cancer cells. It has been demonstrated that glioma cells highly expressed apoptotic markers Bax, caspase-8 and caspase-3 and decreased expression of Bcl-2 when treated by the AM cells, so that their growth were inhibited by induction of apoptosis \([10]\). Along with this mechanism, AM could induce apoptosis in two lines of cancer cells. The viability of cancer cells treated with the amniotic membrane supernatant was significantly decreased by induction of apoptosis signalling pathway and increase of caspase-8 and caspase-3 expression in cancer cells (our unpublished data).

In contrast to the effects of the AM, it has been shown that HSP90 negatively regulates apoptosis through activation of NF-kB and possesses anti-apoptotic effects. Death domain kinase RIP, as a HSP90-associated kinase, allows activation of NF-kB and leads to cell protection against apoptotic death \([26]\). It has also demonstrated that Hsp90 inhibits apoptotic process by binding to APAF-1 (Apoptotic Protease Activating Factor-1) and preventing its cytochrome c-mediated oligomerization \([27]\). Mitochondria-released cytochrome c leads to formation of apaf-1-caspase-9 apoptosis which then activates caspase cascade and induces apoptosis. APAF-1 inhibition by HSP90 alters procaspase 9 activation and prevents apoptosis \([28]\).

Evidence 3

Amniotic membrane could inhibit tumor growth by anti-angiogenic effects. Previous studies reported that the AM secretes substances such as TIMPs (tissue inhibitors of metalloproteinase 1, 2, 3 and 4), collagen XVIII (which converts to endostatin) and thrombospondin-1 (TSP-1) \([29]\) that can inhibit angiogenesis and prevent tumor growth. We recently showed that epithelial cells of the AM prevent angiogenesis through secretion of soluble factors \([30]\). These soluble factors may inhibit angiogenesis by inhibition of hypoxia inducible factor-1α (HIF-1α), as a positive regulator of angiogenesis \([31]\).

HIF-1α is an important client protein of HSP90 which has potential role in inducing angiogenesis. HIF-1α activity depends on environment oxygen. In normoxia, activation of an E3 ubiquitin ligase (von Hippel Lindau protein (VHL)) targets HIF-1α and makes it suitable for proteasomal degradation; so that the amount of HIF-1α reduces in cancer cells. But in low oxygen concentration, VHL function is impaired and leads to HIF-1α accumulation, and then HIF-1α in hypoxic cancer cells is heterodimered by constantly expressed HIF-1β which forms HIF-1α transcription factor. HIF-1 activates VEGF that is an important mediator of angiogenesis and also improves invasion and metastasis of tumor cells by activating MMP-2 \([32]\). It has also been shown that HSP90 has crucial role for HIF-1α stabilization and activation. Inhibition of HSP90 degrades HIF-1α in a VHL independent proteasomal pathway \([33]\). It has frequently reported that HSP90 is very important for transcriptional activity of HIF-1 and heterodimerization of HIF-1 is dependent on HSP90 \([34,35]\).

Furthermore, HSP90 has direct and indirect effects on promoting angiogenesis. Activated HSP90 binds to eNOS and enhances its phosphorylation. Then, NO production of phosphorylated eNOS leads to angiogenesis \([36]\). Moreover, inhibition of HSP90 stimulates degradation of VEGFR-2, a significant receptor of VEGF-A in angiogenesis \([37]\). HSP90 also contributes in expression and secretion of pro-angiogenic cytokines and growth factors from tumor cells \([38,39]\).

Hypothesis

Regarding the provided evidences, we hypothesized that induction of apoptosis, inhibition of angiogenesis and cell cycle arrest in tumor cells mediated by amniotic membrane are through inhibition of HSP90 \((\text{Fig. 1})\). In other words, unknown substances from amniotic membrane inhibit HSP90, which in turn leads to inducing of apoptosis, inhibition of angiogenesis and cell cycle arrest in tumor cells.

Evaluation of hypothesis

After obtaining suitable amniotic membrane, condition medium of cultured AM will be added to cancer cells culture for a specific time to evaluate its effects on cancer cells viability in vitro. To consider if the concentration of the AM condition medium could influence the rate of cancer cells viability, the supernatant of the AM culture would be added to cancer cells culture in dose-dependent manner. In order to find the reasons of changes in cancer cell viability, specific markers of cell death like apoptotic markers could be evaluated. To assess the role of HSP90 in evaluation of our hypothesis, gene expression analysis would be carried out and expression and activation of HSP90 protein, HSP90 client proteins such as cyclin-dependent kinases (CDK 2, 4 and 6) and amount of ATP-bound Hsp90 could be measured.

To evaluate the in vitro effects of the AM condition medium and cells on tumors, animal models would be used by injection of cancer cells to proper tissue of immunosuppressed animals. After appropriate tumor formation, the effects of the AM could be evaluated by injection of the AM culture supernatant or injection of AM cells (epithelial or mesenchymal cells). Then, the tumor size, apoptosis of tumor cells, the rate of angiogenesis and gene expression and protein activation of HSP90 will be evaluated.

Discussion

Recent researches are focusing on molecular mechanisms involved in tumor growth and metastasis to find new methods for cancer therapy. These new therapeutic ideas are based on inhibition of important proteins which their over-expressions lead to tumor invasion and poor prognosis of patients \([40]\). HSP90 is one of these proteins which can protect cancer cells through inhibition of cell cycle arrest and apoptosis, and also enhancement of angiogenesis \([19]\).

Although amniotic membrane, as a natural biomaterial, has been used in many clinical situations, anti-cancer effect has recently been reported to affect angiogenesis of tumor cells. It has also been shown that amniotic membrane supernatant leads to induction of apoptosis, inhibition of angiogenesis and cell cycle arrest. In this paper, we suggested a mechanism which can solely
explain the mentioned effects. HSP90 is common regulator of cell cycle, apoptosis and angiogenesis.

There are some reports which ascribe anti-cancer effects of the AM to stem cells characteristics of amniotic epithelial and mesenchymal cells. Anti-tumor effects of adult stem cells were shown previously. It was reported that human umbilical cord mesenchymal stem cells release nitric oxide and cytokines that inhibit cell growth and proliferation [41]. Inhibition of Akt signaling was also shown by human adult stem cells in some cancer cell lines [42]. It is thought that the AM stem cells can inhibit cancer cells in the same manner, probably through release of cytokotoxic cytokines such as tumor necrosis factor-α, tumor necrosis factor-β, transforming growth factor-β, interferon-γ, IL-2, IL-3, IL-4, macrophage colony-stimulating factor and IL-8 [11]. In addition, some other factors such as granulocyte macrophage colony-stimulating factor, IL-6, neurotrophin 3, CCL18 (a chemokine), macrophage colony-stimulating factor, granulocyte chemotactic protein (GCP-2) and conserved dopamine neurotrophic factors are introduced as mediators of the AM anti-cancer effects [43]. Regulation of the immune cells by AM cells was also demonstrated as cancer inhibition mechanism [44].

In addition to controversial reports about the effects of the mentioned cytokines on cancer cells, it seems these mechanisms are not comprehensive enough to justify the anti-tumor effects of the AM including anti-angiogenesis, cell cycle arrest and inducing apoptosis. Hence, inhibition of HSP90 by the AM unknown ingredients appears more probable than the mentioned mechanisms, which can comprehensively justify the AM anti-cancer effects.

In conclusion our hypothesis demonstrates that anti-cancer effects of the amniotic membrane including cell cycle arrest, induction of apoptosis and inhibition of angiogenesis are mediated by inhibition of HSP90.

Conflict of interest statement

None Declared

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