

# The biological mechanism involved in anticancer properties of amniotic membrane

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## Abstract

The main role of amniotic membrane (AM), or amnion, is to protect the fetus from drying out and create an appropriate environment for its growth. AM is also a suitable candidate for the treatment of various diseases due to its unique characteristics. In recent years, a new line of research has focused on the anticancer properties of amnion and its potential use in cancer treatment. The *in vitro* and *in vivo* studies indicate the anti-proliferative and pro-apoptotic activities, as well as the angioregulatory and immunomodulatory properties of the amniotic membrane. However, the exact mechanism and molecular basis of these anticancer effects of AM are not fully elucidated. This paper presents an overview of the latest findings and knowledge about the anticancer effects of AM and its underlying molecular mechanisms, which is crucial for the application of amnion in cancer therapy.

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## Introduction

Amniotic membrane (AM), or amnion, is an extra embryonic envelope that surrounds the fetus. Amnion is a transparent membrane without nerves, muscles, and lymphatic vessels. It consists of three histological layers, including epithelial, basal, and stromal layers. The stromal layer is divided into compact, fibroblast, and spongy layers (Figure 1).<sup>1</sup> Two types of cells have been isolated from amnion, namely amniotic epithelial cells (AEC) and amniotic mesenchymal stromal cells (AMSC).<sup>1</sup> Both populations express the specific surface and intracellular markers of stem cells, such as stage-specific embryonic antigen-4 (SSEA-4), tumor-related antigen-1-60 [TRA-1-60] and TRA-1-81, and can differentiate into various cell types.<sup>2</sup> Amniotic cells show low to intermediate expression of human leukocyte antigen (HLA)-A, -B, -C, -D, and HLA-QR antigens, without expression of HLA-G.<sup>2</sup> Consequently, these cells do not stimulate immune responses and allograft rejection after transplantation due to lack of immunogenicity.<sup>1-3</sup>

The AM has many properties that make it suitable for clinical use, as it: i) provides an extracellular matrix that allows the attachment and proliferation of cells; ii) stimulates epithelization and inhibits scarring;<sup>4,5</sup> iii) acts as an angiomodulator; iv) synthesizes the natural inhibitors of metalloproteinases;<sup>6,7</sup> v) possesses anti-inflammatory, hemocompatibility, and anticancer properties; vi) is a non-tumorigenic tissue; and vii) has useful mechanical properties.<sup>8</sup> Another advantage of AM is that its use is ethically acceptable because the placenta and AM are usually discarded after childbirth.<sup>8,9</sup>

The first documented use of AM for clinical application dates back to 1910 when Davis used AM for the reconstruction of skin.<sup>1,5,8</sup> AM is now most commonly used in ophthalmology, especially in the reconstruction of the cornea and the ocular conjunctiva, as it promotes the regeneration of the epi-body and inhibits inflammation and scarring.<sup>5</sup> In addition, AM is also used in dermatology for the treatment of burns and chronic ulcers as well as some surgical procedures, such as the prevention of post-surgery adhesions, abdominal surgery, prevention of peritoneal adhesions, gastrointestinal treatment, and reconstruction of the urinary tract.<sup>10</sup> Amniotic membrane works as a scaffold for proliferation and differentiation due to the presence of collagen types I, III, IV, V, and VI, fibronectin, nidogen, elastin, and hyaluronic acid.<sup>1,3,4</sup> Furthermore, there are studies that indicate the anticancer effects of AM, but the mechanism of this effects is yet abstruse and unclear.<sup>11,12</sup>

In this review, we will discuss the anticancer properties of various derivatives of amniotic membrane, as cells or conditioned medium. Moreover, we will highlight the biological pathways that AM uses to carry out anti-carcinogenic activity.

## Hallmarks of cancer

Cancer is the second most common cause of death in humankind. Despite advances in the prevention and treatment of cancer, the number of cancer-related illnesses and various risk factors (smoking, obesity, nutrition, and environmental factors) continue to increase.<sup>13</sup> Tumors are complementary systems that include cancer cells, immune system cells, endothelial cells, and cancer-associated fibroblasts.<sup>14</sup> Hanahan and Weinberg described the essential properties of cancer cells in 2000 and 2011, including: i) the ability of continuous cell division; ii) non-responsiveness to signals that inhibit cell division; iii) avoidance of apoptosis; iv) non-terminal potential for mitosis; v) stimulation of angiogenesis; vi) invasiveness and metastasis; vii) genomic instability; viii) reprogramming of cellular energy metabolism; ix) stimulation of inflammation; and x) avoidance of the immune system.<sup>15,16</sup>

Several studies have aimed to clarify the application of stem cells in cancer therapy.<sup>17,18</sup> Therefore, identification of alternative sources of stem cells with optimal biological proprieties and easy yield or isolation has become a crucial issue in current studies. The AM is a promising alternative source for this purpose.

Previous authors have reported the safe and successful application of cryopreserved amniotic membrane and freshly frozen AM graft for non-melanotic tumors as well as melanoma.<sup>19,20</sup> The use of AM graft has improved the local surgical outcomes by refining healing and reducing scarring<sup>19</sup> and has made possible the excision of wider margins around the tumor.<sup>20</sup>

Tabatabaei *et al.* investigated the potential of human amniotic epithelial cells (hAEC) as a vaccine for cancer prevention and therapy in a mouse model of colon adenocarcinoma. They observed considerable reduction in tumor burden in the tumor-bearing mice immunized with hAEC.<sup>21</sup>

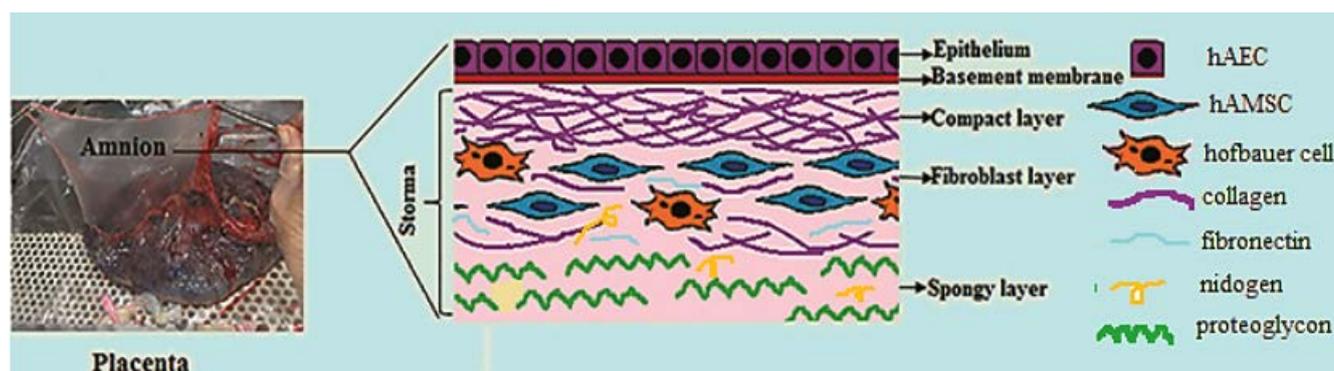


Figure 1. The picture of placenta and the schematic structure of amniotic membrane.

Table 1. The anticancer effect of amniotic membrane cells or conditioned medium and underlying mechanism.

Cell type	Targeted cell line/Cancer type	Cellular effect	Molecular effect	Ref
Amniotic epithelial cells	Human cervical carcinoma (Hela) and breast cancer (MDA-MB-231)	- Decrease of cancer cell viability - Anti-angiogenic - Apoptosis induction	Increase of caspase-3,-8	11
Amniotic mesenchymal cells	Acute myelogenous leukemia (KG1 cells), T-cell leukemia (Jurkat), monocytes from histiocytic lymphoma (U937), Human cervical carcinoma (Hela)	Anti-proliferative	Cell cycle arrest in G0/G1 phase	31
Amniotic mesenchymal cells	Brain tumor (C6-glioma)	- Inhibition of cell migration - Apoptosis induction	---	36
Amniotic epithelial and/or mesenchymal cells	Hepatocarcinoma (HuH7, HepG2, and Hep3B2.1-7)	- Apoptosis Induction - Inducing cell morphology alterations, - Inducing HCC cytotoxicity and cell death	- Reduction the metabolic activity - Increase in cytochrome. C release, BAX/BCL2 ratio, - Increase in caspase 3, caspase 8, and caspase 9	39
Amniotic epithelial cells	T-cell leukemia (Jurkat)	Apoptosis induction	Caspase-3 pathway	43
hAM- protein extract	Prostate cancer (PC3), colon cancer (WiDr, C2BBel, LS1034), pancreas cancer (PANC-1), hepatocarcinoma (HepG-2, Hep3B2.1-7), breast cancer (MCF7, HCC1954), bile ducts (TFK-1)	---	Different inhibit the metabolic activity depend on particular genetic profile of each cell line - Increase of metabolic activity in C2BBel, LS1034, HCC1954 and TFK-1 cell lines. - Reduction of metabolic activity in PANC1, HepG2, Hep3B2.1-7 WiDr and PC3 cell lines	47
hAM- protein extract	Prostate cancer (PC3)	Reduction of cell proliferation	Decrease in gene expression and HSP90 protein expression	75

## Amniotic membrane inhibits cancer cells proliferation

Normal cells accurately control the biosynthetic pathways and release of signaling molecules that stimulate their growth and cause mitotic depletion or inhibit cell growth and prevent their entry into the mitotic division.<sup>22</sup> This maintains the normal architecture and function of the tissue. During development, cancer cells gain the ability to ignore cellular signals that prevent cell division, which leads to the continuation of their mitotic divisions.<sup>23</sup> Cell cycle proteins, in particular cyclins and cyclin-dependent kinases, as well as cell cycle checkpoint proteins constitute potential therapeutic targets in cancer therapy.<sup>24,25</sup> Seo *et al.* reported the anticancer properties of amnion for the first time in 2008. They proposed amnion for cancer treatment owing to its anti-angiogenesis, immunoregulatory, and pro-apoptotic properties.<sup>26</sup> Afterwards, researchers like Kim, Niknejad, Mamede, and colleagues<sup>1,11,27</sup> tested this hypothesis. Some of studies on anti-cancer effects of amniotic membrane cells or its derived conditioned

medium and underlying mechanisms have been summarized in Table 1. The anticancer effects of AM and its cells are due to the release of fatal factors and cytokines for tumor cells, such as macrophage granulocyte colony stimulating factor, neurotrophin-3, transforming growth factor beta (TGF- $\beta$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), tumor necrosis factor beta (TNF- $\beta$ ), C-C motif chemokine ligand 18 (CCL18), protected dopamine neurotrophic factor, macrophage stimulating factor, granulocytic chemotactic protein (GCP-2), brain-derived neurotrophic factor (BDNF), and interleukin-2 (IL-2), IL-4, IL-6, and IL-8.<sup>11,28,29</sup> The anti-tumor effects of these factors have been described and they could participate in the mechanisms involved in the inhibition of cancer cell proliferation.

The inhibitory effect of AM on the proliferation of cancerous cells has been demonstrated *via* different ways, including attenuation of gene expression of certain cyclins (cyclin D2/E1/H) and cyclin-dependent kinase-2 (CDK2), CDK4, CDK6, which promote the progression of the cell cycle, reduction of cell cycle stimulators, and increase of cell cycle suppressors such as p53 and

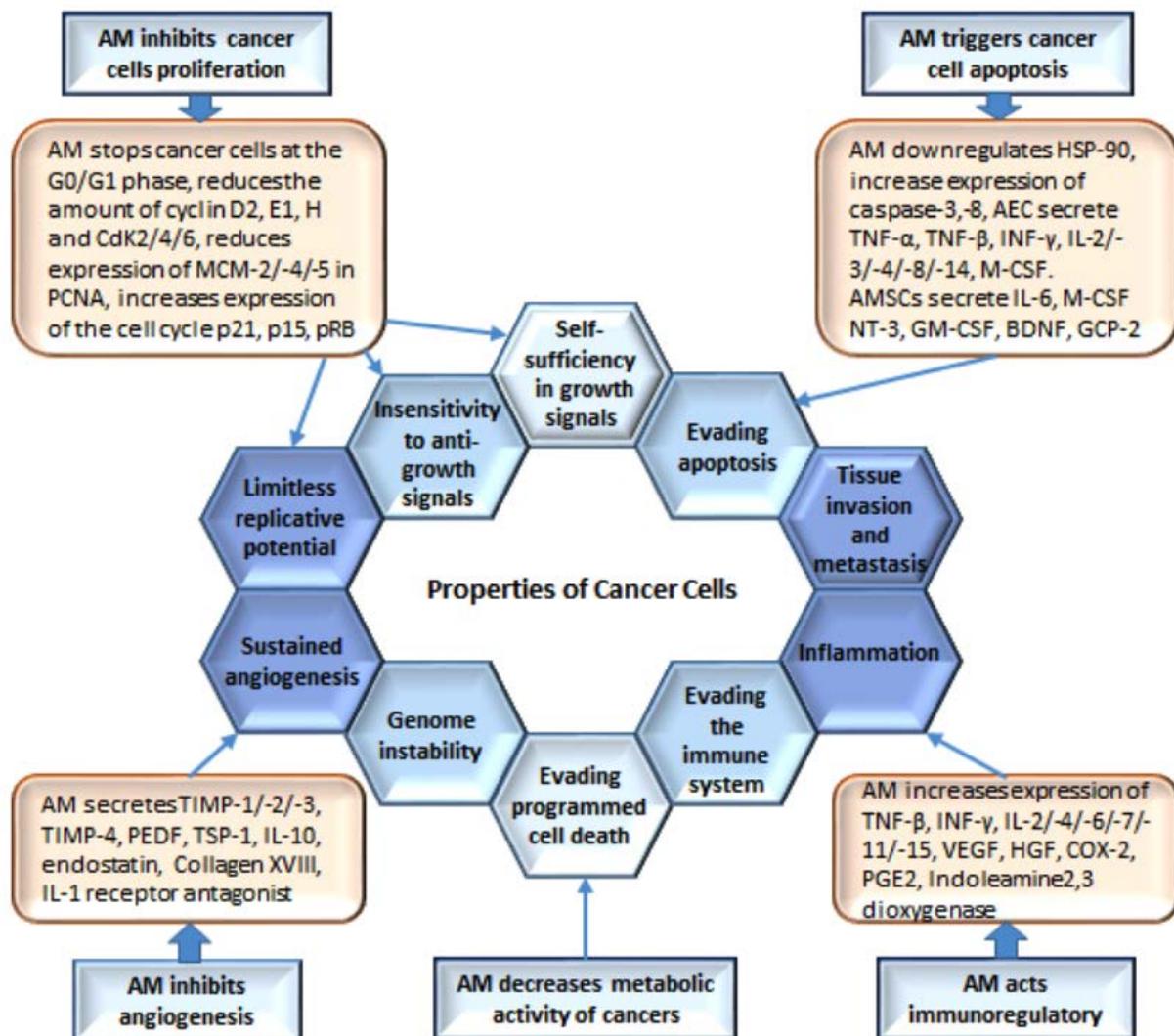


Figure 2. The effect of amniotic membrane (AM) on the properties of cancer cells. AM affects cancer cells through antitumor mechanisms, such as angiogenesis inhibition, cell proliferation inhibition, metabolic activity reduction, apoptosis promotion, and immunomodulatory effect.

retinoblastoma protein (pRB).<sup>29-31</sup> Magatti *et al.* elucidated the cell cycle arrest induction of hematopoietic and non-hematopoietic cancer cells in co-culture with hAMSCs by downregulation of the cell cycle stimulators, such as CDK2, CDK4, CDK6, MCM complex, and proliferative cellular antigen (PCNA), and upregulation of cell cycle inhibitors (cyclin G2, CDK inhibitor N2B, and CDK inhibitor 1A).<sup>31</sup> Furthermore, they reported down-regulation of Cullin-1, which involved in the ubiquitination process, the degradation of different protein like p21 and, consequently, contribute to the block of cell cycle progression. In recent study, increased expression of pRB, which is important for proper cell cycle arrest in G0/G1 phase, while decreased expression of RB-like 1 (p107) were observed in both U937 and Jurkat cells.<sup>32</sup> It is documented that the level of p107 is generally low in quiescent cells and high when cells proliferate. Therefore, these alternations of gene expression in cancer cells after co-culture with hAMSCs indicate the ability of AM in cancer cell proliferation. In other words, amnion is able to stop the excessive mitotic division, which is one of the essential properties of cancer cells.<sup>29,31</sup>

### Amniotic membrane promotes cancer cells apoptosis

Apoptosis is a programmed cell death that is a natural barrier to cancer development.<sup>33</sup> Due to some genetic and chromosomal mutations, natural cells turn into cancerous ones.<sup>34</sup> Many studies have revealed that AM (as its cells or conditioned medium) can stop the proliferation of cancer cells or even trigger their apoptosis.<sup>35-42</sup> The reduced survival of cancer cells under the influence of AM is due to cytotoxic factors excreted by AEC as well as AMSC (Figure 2).<sup>11,12,31,35,43,44</sup> Niknejad *et al.* reported a significant increase in caspase-3 and caspase-8 expression in HeLa and MDA-MB-231 cells after treatment with hAEC-CM.<sup>11</sup> Bcl-2/caspase pathway propounded as another mechanism of AM to induce apoptosis in tumor cells. In one study, Jiao *et al.* showed the inhibition of cell migration and induction of apoptosis by AMSC, leading to a reduced proportion of brain tumors of the gliomas in rats.<sup>36</sup> The authors concluded the decreased ratio of Bcl-2/Bax due to increased expression of apoptotic markers (*e.g.*, Bax, caspase 8, and caspase-3) and decreased expression of anti-apoptotic marker (Bcl-2) after treatment of glioma cells with AM, is responsible for triggering apoptosis in these cells.<sup>36</sup>

Amniotic membrane cells and their conditioned medium induce apoptosis in several cell lines (HeLa cervical cancer cells, hepatocarcinoma cancer cells HepG2, MDA-MB-231 breast cancer cells, Hep3B2.1-7, Hep3B2.1-8, and HuH7, as well as in animal models (glioma in BALB/C mice, breast tumor in BALB/C nu mice, and hepatocarcinoma in BALB/C nu/nu mice).<sup>38-40</sup>

We have previously reported that the conditioned medium from AM inhibits the expression of the HSP90 heat shock protein, which triggers cell cycle inhibition, apoptosis, and the inhibition of angiogenesis.<sup>41</sup> HSP90 prevents apoptosis by activating NF-KB on the one hand and binding to APAF-1 (apoptotic protease activating factor-1) and blocking the caspase9 cascade on the other hand.<sup>42</sup> Additionally, another study has shown that the protein extraction of AM, prepared by homogenization and sonication of AM, triggers the apoptosis of carcinoma cells *in vitro*.<sup>39</sup> The obtained results in that study indicate hAM protein extract is able to induce cell death pathway depend on the biological and genetic profile of the cancerous cell line. The authors reported stimulation of the intrinsic apoptotic pathway (as an increase in cytochrome C release, Bax/Bcl2 ratio, caspase 3, and caspase 9) in HuH7 cell

line, while induction the extrinsic apoptotic pathway (as an increase in caspase 3 and caspase 8) by hAM protein extract in HepG2 and Hep3B2.1-7.<sup>39</sup>

Li *et al.* demonstrated the effect of AECs supernatant on apoptosis of Jurkat cells.<sup>43</sup> They suggested that AECs induced apoptosis through caspase pathways. Their results indicated that AECs express human TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), TNF- $\alpha$ , and Fas ligand (FasL).<sup>43</sup> The role of these factors in participating in apoptosis is well known.<sup>44</sup> The authors demonstrated that FasL plays a major role in AEC-mediated apoptosis.<sup>43</sup>

### Amniotic membrane reduces cancer cells metabolism

For uncontrolled cell proliferation, a change in cellular energy metabolism is essential, which allows the growth and survival of cancer cells. In comparison with normal (healthy) cells, which receive energy primarily by oxidative phosphorylation, cancerous cells mostly generate energy through aerobic glycolysis (*i.e.*, Warburg effect).<sup>45</sup> Such changes in cancer cell vectors allow for increased energy production, sufficient biosynthesis of macromolecules, maintenance of the redox balance, and thus the emergence of tumors.<sup>16,46</sup> Proteins extracted from AM, prepared by homogenization and sonication, affect the metabolism of cancer cells (Figure 2). Mamede *et al.* demonstrated that the protein extracted from AM inhibits the energy metabolic activity in 14 different cell lines and even more than 50% of the metabolic activity in five cell lines (PC3, WiDr, PANC-1, HepG2, and Hep3B2.1-7), as well as in esophagus cancer cells, osteosarcoma, and melanoma.<sup>47</sup> Consequently, they predict that the response of cancer cells to AM is specific to the genetic profile or the type of cancerous cell.<sup>47</sup>

### Amniotic membrane has immunosuppressive effect on cancerous cells

Multiple studies have shown that the immune system can transform normal cells into cancer cells.<sup>44,46</sup> Inflammation in unregulated conditions can induce malignant growth and tumor initiation in the surrounding tissue due to the continuous production of growth factors as well as reactive oxygen species that interact with DNA, resulting in permanent genomic alterations. In addition to the initiation of the tumor, inflammation plays a critical role in tumor progress, malignant transformation, and metastatic dissemination.<sup>46-48</sup> Considering the inflammatory changes in various types of cancer, preventing or reversing inflammation is a decisive approach to cancer control. Numerous reports have presented evidence on the immunosuppressive properties of AM, which can help to maintain fetal-maternal tolerance during pregnancy.<sup>47,49-51</sup> The mother's immune system is challenged with the fetus, which must be tolerated despite the semi-allergen. However, AM is a biological barrier to supporting the homeostatic and tolerant immune milieu required for a successful pregnancy.<sup>36</sup> The AM exerts immunosuppressive effects via various signaling pathways. The amniotic membrane cells (AEC and AMSC) inhibit the differentiation of monocytes into dendritic cells, thereby reducing their ability to stimulate the T lymphocytes. Both the AM cells and conditioned medium of AM inhibit peripheral blood mononuclear cell proliferation (PBMC).<sup>52</sup> AM downregulates the expression of cell surface markers, such as CD80, CD86, and MHC II, which are

modulators of the immune response.<sup>53</sup>

Various studies have shown that cancerous cells often increase the secretion of inflammatory chemokine IL-8 in response to chemotherapeutics or other stressful environmental conditions (e.g., hypoxia).<sup>54</sup> In this way, the IL-8 signaling also influences the proliferation,<sup>55</sup> migration, and cancer cell invasion<sup>56</sup> and even helps cancer cells to avoid apoptosis.<sup>50</sup> Magatti *et al.* reported that AEC and AMSC inhibit the expression of IL-8 in co-culture with dendritic cells.<sup>57</sup> It is assumed that this effect of AM can contribute to limiting the development of tumors by inhibiting inflammation.<sup>57</sup> The AEC synthesizes the migration inhibitory factor, which is an inhibitor of the migration of macrophages.<sup>58</sup> Furthermore, AM cells can modulate the T lymphocyte and immune lymphocytes *in vitro*. These cells, in particular, capable of inhibiting the allogeneic proliferation of lymphocytes.<sup>59,60</sup> There is evidence that transplanted cryopreserved AM induces the apoptosis of macrophages, monocytes, and neutrophils; decreases the infiltration of macrophages, neutrophils, and lymphocytes; and promotes the polarization of M2 macrophages.<sup>37,38</sup> Also, AM reduces secreted pro-inflammatory cytokines, such as TNF- $\alpha$  and IL-6, while it upregulates anti-inflammatory cytokines, such as IL-10, which, in turn, decreases the inflammation.<sup>61</sup> AM cells inhibit the synthesis of inflammatory cytokines without any inflammation. This could prevent the access of growth and proangiogenic agents and enzymes that would modulate the extracellular matrix into the tumor microenvironment, thereby inhibiting/limiting further development of the tumor.<sup>62</sup> Additionally, the conditioned medium (CM) delivered from amniotic cell culture has shown an anti-proliferative effect on lymphocytes, thus providing evidence of paracrine-mediated immunosuppressive activity.<sup>51</sup>

Interestingly, CM derived from amniotic cells plays a role in modulating the activation of human microglia (the resident immune cells in the brain and spinal cord). *In vitro* data showed in microglia co-cultured with hAMSCs, production of TNF- $\alpha$  inflammatory cytokine was suppressed.<sup>51</sup>

### Amniotic membrane has angiomodulatory effect on cancerous cells

Tumor cells, due to their high growth rate, need a more extensive source of nutrients and oxygen and a more intense discharge of metabolic waste and carbon dioxide compared to normal tissues. Therefore, tumor progression is associated with neovascularization and angiogenesis.<sup>63</sup> In this context, antiangiogenic agents that prevent the emergence of new blood vessels could block the supply of nutrients and oxygen to the tumor. Studies have revealed AEC and extracellular matrix proteins of AM, e.g., collagen IV, fibronectin, and collagen VII, are involved in inhibition of angiogenesis in cancer tissues.<sup>64-66</sup>

AM also secretes pigment epithelium-derived factor (PEDF), tissue inhibitor metalloproteinase-1 (TIMP-1), TIMP-2, TIMP-3, and TIMP-4, thrombospondin-1 (TSP-1), IL-1 receptor antagonists (IL1RN), and IL-10, which are able to trigger the antiangiogenic process.<sup>12,30,63-67</sup> Matrix metalloproteinases (MMPs) participate in the destruction of extracellular matrix and can therefore facilitate angiogenesis by helping cancer cells to attack the basal lamina.<sup>68</sup> The TIMPs secreted by AM have ability to obstruct the function of MMPs.<sup>67</sup> On the other hand, IL-10 blocks angiogenesis by inhibiting the secretion of some MMPs and promoting the secretion of TIMP-1.<sup>67</sup> IL1RN, another protein secreted by AM, through impeding the transcriptional impact of IL-1 on cyclooxygenase-2 diminishes the expression of some potent angiogenic substances

like VEGF and IL-8.<sup>68</sup>

On the contrary, it has been demonstrated that angiogenic factors, such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), heparin-binding EGF, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), IL-8, IL-6, and angiopoietin-2, are secreted by AM.<sup>49,69</sup> In a recently published article, Ling *et al.* demonstrated transplantation of human amniotic mesenchymal stem cells (hAMSC) in rats with premature ovarian insufficiency (POI). They reported CM derived from hAMSC (hAMSC-CM) has an effect on POI mainly through a paracrine mechanism.<sup>35</sup> In fact, hAMSCs mediate angiogenesis through secretion of growth factors, including FGF2, insulin-like growth factor 1 (IGF-1), HGF and VEGF, thereby reducing ovarian injury, regulating follicle growth, and improving ovarian function.<sup>35</sup> According to our previous findings, the angiogenic/antiangiogenic effect of amnion is a side-dependent manner. While the epithelial side of the amnion inhibits angiogenesis, the mesenchymal side of amnion induces angiogenesis through the release of angiogenic factors or the differentiation of its mesenchymal cells into endothelial cells.<sup>64</sup>

### Clinical trials of amniotic membrane in cancer therapy and its challenges

According to the anticancer properties of amnion mentioned above, as well as its availability, cost-effectiveness, non-immunogenicity and non-tumorigenicity, this tissue and its derived cells can be considered suitable and reliable sources for cancer treatment.<sup>1,7-9,20</sup>

AM grafts may be useful as adjunctive treatment in patients with cancer. In this regard, a completed study reported on clinicaltrials.gov is *Use of Human Dehydrated Amnion/chorion (DHACM) Allograft in Partial Nephrectomy*.<sup>70</sup> In this study, which the impact of DHACM allograft to facilitate the recovery of renal function in patients with kidney cancer after nephrectomy was investigated.<sup>70</sup> There is another completed clinical trial (NCT03515954) that AM graft was secure and attached to the conjunctiva and sclera using fibrin glue in conjunctival neoplasms condition.<sup>71</sup> However, results of two recently mentioned studies are not currently available. One clinical trial on *Randomized AminoFix Study during Radical Prostatectomy* is withdrawn.<sup>72</sup> There is only one completed clinical trial with available results about AM application in cancer condition. This phase II clinical study, investigated by Sanoj Punnen at the University of Miami, and carried out on patients with prostate cancer.<sup>73</sup> In the study, the neurovascular node that remains after prostatectomy was covered with dehydrated human AM allograft, and then the level of prostate specific antigen (PSA) in the blood was evaluated every 3 months for the first 12 months after surgery. After this, the patients were followed annually with PSA measurements and an assessment of any secondary therapies for 5 years post-surgery.<sup>73</sup> In addition to PSA measurements, behavioral changes (the urinary leakage and erectile function) of subjects were evaluated in 140 patients, including 70 men in the control group (patients without AM allograft) and 70 men in the experimental group (patients with AM placement). The results reported on ClinicalTrials.gov.<sup>73</sup> Certainly, future and similar research will significantly develop our knowledge of the anticancer effects of AM and thus may extend the range of clinical AM application.

However, there are some points to be considered when using amnion in the clinical setting. First, data suggest that AECs with their net anti-angiogenic and anti-proliferative effect on cancerous cells are a more appropriate source than AMSCs for the cell thera-

py of angiogenesis-dependent tumors.<sup>66</sup> Second, due to the heterogeneity of the amniotic epithelial cells, it is important to know which region of the tissue (placental AM or reflected AM) to use.<sup>74,75</sup> Finally, providing fresh amniotic cells for therapeutic purposes is one of the clinical application challenges. Hence, it is recommended that these cells be preserved to resolve this problem and also to have a ready-to use source for cancer cell therapy.

Although studies indicate that amniotic membrane is a promising source for cancer cell therapy, further *in vitro* and *in vivo* studies are needed to translate cancer therapy by amnion into clinical applications.

## Future perspective

Nowadays, AM has wide range clinical applications, especially in tissue engineering and regenerative medicine.<sup>4-9</sup> In this context, several companies manipulated amnion to make it more suitable for therapeutic use, and allocated a large market share. The major commercial product include amniotic fluid, amniotic membrane graft, amniotic membrane extract, amniotic membrane transplant, and amniotic cytokine extract.<sup>76</sup> Although these products have not FDA approved, they are widely used for tissue regeneration. Considering anti-cancer properties of amnion, AM graft can be useful for treatment some type of cancers like conjunctival and skin cancer.<sup>20</sup> AM derivatives can be also used as a vaccine for cancer prevention as previously tested in the animal model.<sup>20</sup> Exosomes derived from AM cells may provide a new opportunity in cancer treatment exploiting their delivery function.<sup>77</sup> These nanovesicles may effectively transfer antitumor drugs or RNAs in the context of gene therapy to reduce the stimulatory effects of these drugs on the immune system and the hydrophilic properties that facilitate their passage through cell membranes.<sup>78-80</sup> Translating treatment from lab to clinic requires standardization and increased the efficacy of these AM commercial products. Therefore, with sufficient standards of preservation and production of AM, and well-designed clinical trials, AM and its derivatives are suitable treatment options for cancer treatment in the future.

## Conclusions

AM is now most commonly used in ophthalmology and dermatology. However, the number of studies exploring the possibilities of using AM in other clinical areas is increasing. Since the scope of AM in the case of potential treatments or prevention of cancer is a relatively new area, there are no completed clinical studies in this field. The results of preclinical studies *in vitro* and *in vivo* show that AM has selective cytotoxic effects on various types of cancer cells but does not affect normal cells. This is one of the most important advantages of the use of AM in the treatment of cancer. In addition, due to the anti-inflammatory, anti-fibrotic, pro-apoptotic, and antiangiogenic effects of AM, it could be used as a novel, safe, and inexpensive substance with fewer side effects for cancer treatment in the future and achieve desired effects. For this purpose, further studies are needed in order to elucidate the molecular mechanisms, identify the nature of factors involved in the anticancer effects of amnion, and translate cancer therapy by amnion into clinical applications.

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