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Review Article

Exosomes and Cancer: The Bio Informational Reprogramming Therapy for Malignant Cells

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ABSTRACT

Exosomes are vesicles that carry nanoparticles that play important roles in cell-to-cell communication. They are currently being tested for their potential as therapeutic agents for various degenerative diseases including cancer. This is due to the rationale that these nanoparticles can transfer informational biomolecules, and subsequentially cause metabolic and physiological changes. Also, these vesicles can be used as a drug delivery system and be very effective at reducing toxicity and increasing bioavailability of therapeutic molecules and drugs. Exosomes were first thought as a waste cell product. Current research, has demonstrated that these particles can serve as cancer biomarkers, modulate the immune system, cause re-differentiation in cancer cells, and apoptosis. This review emphasizes on particular capabilities of specific exosomes that can potentially be used for cancer therapy, especially as an informational reprogramming therapy for malignant cells.

Keywords: Exosomes; Cancer; Informational reprogramming

INTRODUCTION

In 2018, it was estimated that over 18.1 million new cancer cases will arise and 9.6 million cancer deaths will result [1]. Despite some therapeutic advancement in recent decades, clinical outcomes of cancer remain poor [2]. To try to solve this major issue, new therapeutic avenues are avidly sought. We will examine the value of the exosomes as an anticancer tool. Exosomes, which were described in 1983 [3], are vesicles that contain nanoparticles that are secreted by multiple types of cells. They are tiny membrane bound vesicles that play an important role in extracellular communication [2]. Exosomes, travel through all the body and carry very specific signals to cause a biological action on a tissue. For example, they have the capacity of regulating the expression of certain proteins capable of causing tumor growth suppression. These nanoparticles are constantly found in biological fluids like plasma, urine, saliva, breast milk, synovial fluids and others [4].

At the moment, considerable research is in progress to explore the physiology and behavior of these particles. There is emerging evidence indicating that exosomes can load unique cargos, including micro RNA, proteins and nucleic acids [5]. Certain exosomes derived from cancer cells can be considered cancer biomarkers and be potentially used in the early diagnosis of this disease. Moreover, in cancer immunotherapy exosomes have promisingly been employed since they contain the necessary immunogenic antigens to stimulate NK cells and T-lymphocytes [6]. In addition to these actions, we would like to propose yet a further mechanistic possibility for certain specific exosomes. We propose Umbilical Cord Stem Cell Derived Exosomes (UCSCDE) as providers of the needed or lacking cellular information in malignant cells to either re-differentiates or induce apoptosis. We believe that this biological information reprogramming can be achieved by specific exosomes that carry the needed differentiation information. This information may be capable of providing the necessary negative entropy (negentropy) to overcome malignant cellular entropy disarray and restore the normal cellular organized state.

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EXOSOMES FORMATION AND STRUCTURE

Process of exosome formation is explained by two mechanisms. The formation of Intraluminal Vesicles (ILVs) within Multivesicular Endosomes (MVEs) (left). Subsequently, the membrane of MVE fuses with the plasma membrane and release of ILVs at which point are considered exosomes. Similarly, vesicles can also be formed by creating an external vesicle directly (right) as shown in Figures 1 and 2.

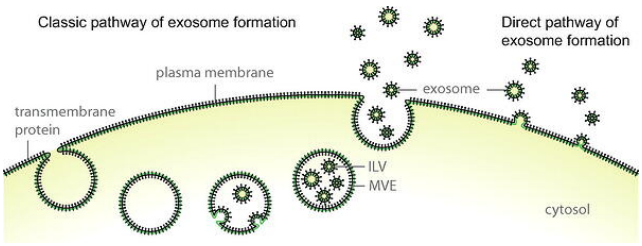

Figure 1: Classic Pathway of Exosome Formation.

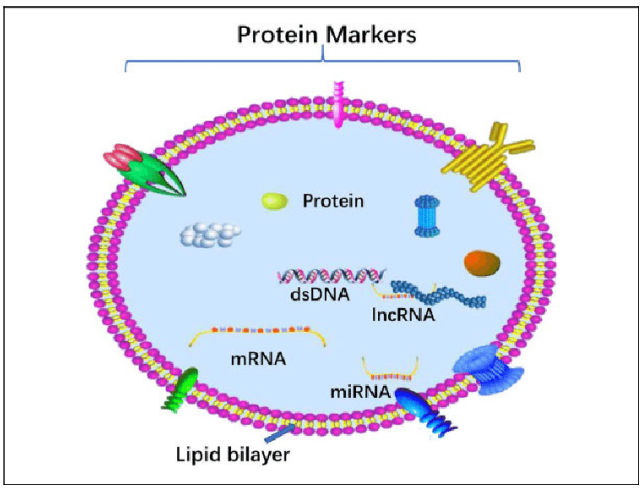

Figure 2: G-Protein Couple Receptor (GPCR) Cycle.

The structure and contents of exosomes consit of a lipid bilayer membrane similar to its originating cell in terms of its transmembrane proteins and receptors. Its contents can be transferred to their target cell, include a variety of RNA species incluing messenger, mitochondrial, non-coding RNA, DNA, nucleic acids and a wide range of different proteins.

EXOSOMES AND CANCER DIAGNOSIS

Scientists and health professionals are taking advantage of the role of exosomes as biomarkers for the diagnosis of diseases. Multiple studies have proven that patients suffering from cancer can possess unique types of exosomes (Oncosomes). Also, it has been discovered that exosome concentrations are elevated in the systemic circulation of patients with various types of cancer [7]. Because of this, exosomes can be used to diagnose cancer, even at an early stage. This can be accomplished by taking a biological fluid sample of a patient and isolating the exosomes from cancer cells. At the moment, there are multiple ways to isolate exosomes. However, the golden standard is differential ultracentrifugation, it accounts for 56% of all exosome isolation techniques employed by users in exosome research [8].

The most common source for exosomes is the blood [5]. Liang et al. worked with blood samples of patients with a specific lung cancer [9]. They isolated from the samples Platelet-derived Extracellular Vesicles (P-EVs). The study revealed that patients with cancer displayed significantly increased levels of P-EVs compared to healthy patients [9]. This states that P-EVs might be useful as cancer biomarkers. Also, they can be analyzed to create future cancer treatments. Another source of acquiring and analyzing exosomes is from urine. Glomeruli can filtrate only very small particles. Since exosomes are basically nanoparticles, they can be easily filtrated through the membrane of the glomeruli and subsequently be excreted along with the urine. Some studies currently involve the analysis of cancer biomarkers by taking samples of urine. On the other hand, in 2012 a research reported that patients with Esophageal Squamous Cell Carcinoma (ESCC) had higher levels of exosomal miR-21 in serum than those with benign diseases [10]. Exosomal miR-21 expression was correlated with presence of metastasis, tumor progression and aggressiveness. It was concluded that exosomal miR-21 may be a useful tool to monitor ESCC progression and measure the effectiveness of treatment in patients.

The presence of exosomes in blood and other biological fluids can open the door for a better diagnosis of cancer since they can capture tumor status, development or even metastasis. They could function as an easy access cancer biomarker. A liquid biopsy containing exosomes could be added as a better non-invasive procedure for patients. We should state that these exosomes are derived from cancer cells (oncosomes), thus containing information to favor cell division and growth, and not cell differentiation as the ones we are proposing as anticancer therapy.

EXOSOMES IN CANCER THERAPY: MULTIPLE ROLES

Exosomes as a drug and molecule carrier in cancer therapy

Since exosomes are very small particles, have minimal toxicity and can be able to penetrate the membrane of a cell easily, they could function as a drug delivery tool in cancer therapy. There are many ways molecules can be implemented on exosomes such as sonication, direct mixing, electroporation and others [11]. Exosomes can deliver proteins, molecules and nucleic acids that help cause a change on a cell. However, to be able to understand the potential of exosomes as drug delivery vesicles, some criteria should be taken in consideration such as size, shape, surface charge, and others [11].

There have been multiple researches of exosomes as a nanoparticle drug delivery system in the last years. In 2016, researchers packed exosomes with curcumin [12]. Curcumin is an anti-inflammatory agent found in Curcuma longa which can be used to treat cancer [13]. However, supplementing curcumin by itself results inefficient since it lacks bioavailability [14]. The former research proved that exosomes isolated from curcumin pretreated H1299 cells can possess anti-cancer effects by TCF21 upregulation [12]. In addition, it was stated that since curcumin was transported in exosomes, it improved the bioavailability in patients.

Moreover, chemotherapy has been characterized for causing toxicity and other harsh side effects on patients. Doxorubicin is a chemotherapy drug approved by the FDA that is commonly used due to its efficiency in fighting multiple types of cancer with good response rate and overall survival [15]. On the other hand, this drug is known to cause apoptosis and necrosis in healthy major organs [16]. A research conducted in 2014, tested the capacity of exosomes as a drug delivery system, with doxorubicin [17]. The results showed that when doxorubicin was implemented on exosomes it inhibited cell proliferation on various tumor cell lines more efficiently than doxorubicin alone. Also, high targeting ability and less toxicity was confirmed. It can be deduced that exosomes possess the capacity of acting as a drug delivery system and can be able to cause less side effects once supplemented on patients.

Exosomes as immune enhancers

Initially, exosomes were taught just to be a waste product of cells. Further research then discovered that they were involved in the mechanisms of direct cell to cell communication [18]. Exosomes derived from specific tissues can be an important component in modulating the immune system and other biological processes [19]. Due to these multiple beneficial functions that exosomes do in tissues and cells; they have been recently implemented in regenerative medicine. The ability of exosomes to fight cancer has been tested in humans and mice. Multiple researches has proven the capacity of tumor inhibition by providing specific exosomes to patients.

Immunotherapy is a treatment that stimulates the immune system to fight multiple diseases, such as cancer. In recent years, exosomes have been experimentally employed as a cancer immunotherapy treatment. Tumor cells are known to release specific types of exosomes. These exosomes have been discovered to be implicated in the regulation of the adaptive immune system. They can function as a source of tumor antigens that T-cells can subsequently recognize. Interestingly, a study presented that exosomes derived from human malignant effusions contained MHC class I molecules and tumor antigens [20]. These two latter molecules mentioned are very important for stimulation of immune cells that can help in tumor growth inhibition. Moreover, tumor inhibition has been detected after *in vivo* administration of exosomes holding tumor antigens, the mode of action may involve stimulation of CD4+ and CD8+ T cells [6]. These T-cells are known to cause apoptosis to tumor cells.

Exosomes as cellular information providers or biological reprogrammers

The composition of exosomes has been studied in the last years. Thousands of different proteins have been identified inside exosomes. These nanoparticles have specific nucleic acids like mRNA that have been shown to be able to transfer their cargo to recipient cells and induce phenotypic changes [21]. Exosomes from different stem cells and from stem cells at different developmental stages may carry unique timely bio-information. Exosomes contain proteins and nucleic acids that directly reflect the metabolic state of the cells from which they originate. Homeostasis relies heavily on effective cell to cell communication. Local and systemic transfer of information seems plausible. Exosomes are pleiotropic in nature and are capable of producing multiple signal transduction cascades. Communication depends on an efficient crosstalk between the specific exosome and the target cell. This paracrine mechanism can mediate cell to cell communication *via* direct receptor stimulation of target cells and the horizontal transfer of genetic material to cells avidly to receive this bio-information. Using exosomes for cancer therapy may provide many benefits. First, they penetrate the cell membrane without difficulty, due to their small size. Secondly, exosomes can be isolated and inserted a specific drug or molecule so it can be sent to a target cell and cause a desired effect. Third, exosomes generally maintain their function once injected in the body. A report in 2015 tested the properties of miR-134 derived from exosomes of patients with Triple-negative breast cancer (TNBC) [22]. The study detailed the therapeutic potential that miR-134 has as a tumor suppressor. It was confirmed that miR-134 reduced TNBC aggression and increased drug sensitivity. MiR-134 in combination with cisplatin, a chemotherapy drug, increased cellular sensitivity to cisplatin-induced apoptosis. This demonstrates that miR-134 may have the potential as an onco-suppressor when used in combination with chemotherapy drugs. Micro RNAs may be a rapid way to regulate gene expression. These micro RNAs are candidates for post-transcriptional regulation and induction of epigenetic changes in the recipient cells, that may be furtherly enhanced by photo-biomodulation, mitochondrial cofactors, oxygen and a ketogenic diet [23].

On the other hand, Mesenchymal Stem Cells (MSC) are found in multiple tissues. They are in charge of tissue homeostasis and differentiation. Research has proven that they can help in tumor suppression. One of the reasons MSC have this ability is due to the specific exosomes they secrete. Exosomes derived from MSC contain multiple cargoes and proteins that may control different relevant metabolic pathways of malignant cells. In 2012, researchers evaluated if exosomes from MSC of human bone marrow can inhibit *in vivo* and *in vitro* growth of multiple tumors. The study revealed that exosomes secreted from MSC of human bone marrow inhibited cell cycle progression and induced apoptosis in various types of cancer cells like hepatoma, ovarian tumor and Kaposi's sarcoma [24].

Another method that is emerging in cancer therapy is cell reprogramming. This approach refers to any intervention that transform cancer cells into terminally differentiated cells [25]. Tumor cells in order to differentiate need to go through cell regulation pathways. These pathways can be controlled by multiple cell differentiation factors. For example, exosomes derived from mesenchymal stem cells can function as these differentiating factors, because these particular instructions are embedded in them. In 2005, researchers isolated Stem Cells Differentiation Stage Factors (SCDSF) that have been proven to inhibit tumor growth *in vivo* and *in vitro* and implemented them into patients with advanced Hepatocellular Carcinoma (HCC) [26]. Results showed improvements for patients with intermediate-advanced HCC. Also, it was stated that this treatment was easy to apply and had negligible side effects for the patients. One of the main concerns of the current

conventional cancer therapies utilized nowadays is the harsh side effects it produces on patients. Exosomes therapy could overcome this concern and possibly cause better clinical outcomes.

UMBILICAL CORD STEM CELL DERIVED EXOSOMES AND CANCER

Stem cells are found and can be isolated in multiple places of our bodies, such as adipose tissue, bone marrow and the umbilical cord. Some functions of these cells are to replace damaged cells or to cause differentiation. Stem cells are packed with the necessary information, including exosomes, to cause an action on another cell. In example, apoptosis can be induced in cancer cells due to the blocking of certain pathways by stem cells.

human Umbilical Cord Stem Cells (hUCMSCs) are unique stem cells derived from Wharton's jelly, which results promising for therapy due to multiple advantages they can possess [25]. Another finding about hUCMSCs derived from Warton's jelly is that compared to other mesenchymal stem cells, they possess a unique transcriptome that shows expression of pro-apoptotic and anti-cancer genes [27]. In 2009, a study proved that administration of intravenously naïve hUCMSC for three weeks significantly attenuated tumor growth in a xenotransplant rat model in which human breast carcinoma was induced [28] and in another study it was observed that exosomes from umbilical cord derived MCS inhibited growth and induced apoptosis of glioblastoma cells *in vitro*. They also corroborated it by *in vitro* studies. Researchers stated that co-cultured hUCMSC produced factors that attenuated the cancer cell growth. Furthermore, a study in 2012 evaluated the role of human wharton's Jelly Stem Cell (hWJSC) extracts in three different cancer cell types [29]. All cancer cell lines showed cell shrinkage and blebbing. It was also noted that the degree of anticancer activity varied between the cancer cell lines. These findings can open a door to new possible therapeutic strategy for cancer. Exosomes can also be obtained from amniotic fluid which can probably provide a higher yield.

EMBRYONIC STEM CELLS FACTORS AND CANCER

Embryonic Stem Cells (ESC) are pluripotent cells. This signifies that they can differentiate into any type of cell with the correct signaling. They can produce a wide range of cells, from a muscle to a nerve cell. Recently, ESCs have been implemented commonly to replace damaged tissues in patients. Also, they can be very convenient since these cells can be grown *in vitro* and produce a large quantity of them. These reasons indicate that ESCs have therapeutic potential.

It has been stated that cancer development can be prevented during embryonic life due to the factors present on the embryonic cells [30]. These factors can be contained in exosomes from these particular cells. In addition, it has been proven that when different tumor cells are implanted in embryos in the period of organogenesis they become differentiated, however if these tumor cells are implanted in adult animals, they cause tumor development [31]. This indicates that the embryo stage is crucial in determining cancer differentiation. In 2019, researchers observed that some factors from Zebrafish embryos during a specific developmental phase can inhibit proliferation of breast cancer cells, and its migrating capabilities [32]. In addition, it has been discovered that Zebrafish Embryonic Cells contain apoptosis-inducing proteins that can act on colon cancer cells [33] and in another study all the kind of factors taken from Zebrafish embryo after the beginning of stem cell differentiation were identified by using a Liquid Chromatography–Mass Spectrometry (LC-MS/MS) analysis, after the in-gel digestion procedure [31]. Identified proteins, which represent 98% of the molecules isolated from SCDF (the remaining 2% of the molecules is represented by nucleic acids) include multiple form of yolk protein vitellogenin, heat shock protein (e.g. HSP8 and HSP70) which are important for the immune response, proteins able to regulate the metabolism of the mitochondria etc. These proteins are implicated in many pathways as in signaling cell cycle regulation, protein trafficking, chaperoning, protein synthesis and degradation. It was confirmed that these proteins do have a reprogramming or apoptotic effect on cancer cells because they act regulating a transcriptional activation of p53 or a post-translational modification of the Protein of the retinoblatoma (pRb), able to reprogram or to address the cancer cells to an apoptotic pathway [34,35]. In addition in order to ascertain if these embryonic factors could synergistically/additively interact with 5-Fluorouracil (5-Fu), whole cell-count, flow-cytometry analysis and apoptotic parameters were recorded in human colon cancer cells (CaCo2) treated with Zebrafish Stem Cell Differentiation Stage Factors (SCDSF 3 μg/ml) in association or not with 5-Fu in the sub-pharmacological therapeutic range (0.01 mg/ml). Cell proliferation was significantly reduced by SCDSF, meanwhile SCDSF+5-Fu leads to an almost complete growth-inhibition. SCDSF produces a significant apoptotic effect, meanwhile the association with 5-FU leads to an enhanced additive apoptotic rate at both 24 and 72 hrs. SCDSF alone and in association with 5-Fu trigger both the extrinsic and the intrinsic apoptotic pathways, activating caspase-8, -3 and -7. SCDSF and 5-Fu alone exerted opposite effects on Bax and Bcl-xL proteins, meanwhile SCDSF+5-Fu induced an almost complete suppression of Bcl-xL release and a dramatic increase in the Bax/Bcl-xL ratio [36]. These data suggest that zebrafish embryo factors could improve chemotherapy efficacy by reducing anti-apoptotic proteins involved in drug-resistance processes. This information is congruent with other studies which demonstrate that differentiation factors can possess epigenetic regulators that are able to regulate cancer cells by activating new pathways [37]. It can be concluded that embryonic stem cells can possess the necessary information that causes differentiation in cancer cells. In addition, it was observed in another study that exosomes from umbilical cord derived MCS inhibited growth and induced apoptosis of glioblastoma cells *in vitro*. These embryonic environments have the intrinsic ability to epigenetically program or reprogram cellular states during development or malignancy. Cancer cells seem to retain a level of plasticity to respond to embryonic differentiation signals that may induce growth arrest and loss of the malignant phenotype. This growth arrest is associated with the upregulation of cell cycle inhibitors and key signaling pathways involved in cell proliferation. Moreover, MCS

exosomes were shown to express functional respiratory complexes which may promote aerobic ATP synthesis restoration in cancer cells.

CONCLUSION

Exosomes are very important vesicles containing nanoparticles for intercellular communication. Due to their ability to pass bio-information to other cells, they can perform multiple functions in the tissues. Cancer treatment needs to be improved by increasing effectiveness and specificity. Research has proven that exosomes can be very specific. They carry multiple cargos, proteins, micro mRNA and nucleic acids that can cause a specific action in a cell. For example, exosomes have the suitable immunogenic molecules that are needed to stimulate T-cells in order to cause apoptosis to cancer cells. In addition, exosomes can acquire important factors from different types of stem cells and cause differentiation or apoptosis in cancer cells. These factors are the most important part, since they are the ones that can cause beneficial phenotypic modifications to the cells.

The therapeutic ability of exosomes to fight cancer can be very promising. Exosomes are relatively easy to use, specific and non-toxic. These characteristics demonstrate great potential for implementation of exosomes as a therapy for cancer. Cancer cells seem not to be irretrievably locked in the malignant state, in the presence of embryonic differentiation control systems they could return to normal. This reprogramming of the cancer cell epigenome may be accomplished by modifying DNA methylation and histone modification in the promoter region of the silenced tumor suppressor genes, increasing p53 expression resulting in tumor growth reduction. However, more research needs to be done to fully understand, specifically select and effectively apply this differentiation promoting exosomes. We need to better understand the physiological effect of different exosomes of diverse developmental stages and arising from cells of different tissues. It's critical to know more about the composition of these nanovesicles so we can understand better how to use them as cancer treatment.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clini. 2018;68(6):394-424.
2. You B, Xu W, Zhang B. Engineering exosomes: a new direction for anticancer treatment. Am J Cancer Res. 2018;8(8):1332-1342.
3. Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: Selective externalization of the receptor. Cell. 1983;33(3):967-978.
4. Brinton LT, Sloane HS, Kester M, Kelly KA. Formation and role of exosomes in cancer. Cell Mol Life Sci. 2015;72(4):659-671.
5. Huang T, Deng CX. Current Progresses of Exosomes as Cancer Diagnostic and Prognostic Biomarkers. Int J Biol Sci. 2019;15(1):1-11.
6. Wang Q, Ding X , Zhen F , Ma J , Meng F. Remedial Applications of Exosomes in Cancer, Infections and Diabetes. Acta Poloniae Pharmaceutica. 2017;74(2):313-320.
7. Kalluri R. The Biology and function of exosomes in cancer. J Clin Invest. 2016;126(4):1208-1215.
8. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in Exosome Isolation techniques. Theranostics. 2017;7(3):789-804.
9. Liang H, Yan X, Pan Y, Wang Y, Wang N, Li L, et al. MicroRNA-223 delivered by platelet-derived microvesicles promotes lung cancer cell invasion via targeting tumor suppressor EPB41L3. Mole Cancer. 2015;14(1):58.
10. Tanaka Y, Kamohara H, Kinoshita K, Kurashige J, Ishimoto T, Iwatsuki M, et al. Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma. Cancer. 2012;119(6):1159-1167.
11. Kibria G, Ramos EK, Wan Y, Gius DR, Liu H. Exosomes as a Drug Delivery System in Cancer Therapy: Potential and Challenges. Mol Pharm. 2018;15(9):3625-3633.
12. Wu H, Zhou J, Zeng C, Wu D, Mu Z, Chen B, et al. Curcumin increases exosomal TCF21 thus suppressing exosome-induced lung cancer. Oncotarget. 2016;7(52):87081-87090.
13. Hewlings SJ, Kalman DS. Curcumin: A Review of Its' Effects on Human Health. Foods. 2017;6(10):92.
14. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007;4(6):807-818.
15. Carvalho C, Santos RX, Cardoso S, Correia S, Paulo O, Maria S, et al. Doxorubicin: the good, the bad and the ugly effect. Current Medicinal Chemistry. 2009;16(25):3267-85.
16. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 2013;65(2):157-170.
17. Tian Y1, Li S, Song J, Ji T, Zhu M, Anderson GJ, et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials. 2014;35(7): 2383-2890.
18. Bang C, Thum T. Exosomes: New Players in cell-cell communication. The Inter J Biochem & Cell Biol. 2012;44(11):2060-2064.
19. Baquir B, Hancock RE. Exosomes, your body's answer to immune health. Ann Transl Med. 2017;5(4):81.
20. Andre F, Schartz NE, Movassagh M, Flament C, Pautier P, Morice P, et al. Malignant effusions and immunogenic tumour-derived exosomes. The Lancet. 2002;360(9329):295-305.
21. Tickner JA, Urquhart AJ, Stephenson SA, Richard DJ, O'Byrne KJ. Functions and therapeutic roles of exosomes in cancer. Front Oncol. 2014;4:127.
22. O'Brien K, Lowry MC, Corcoran C, Martinez VG, Daly M, Rani S, et al. miR-134 in extracellular vesicles reduces triple-negative breast cancer aggression and increases drug sensitivity. Oncotarget. 2015; 6(32):32774-89.
23. Gonzalez MJ, Seyfried T, Nicolson GL, Barclay BJ, Matta J, Vazquez A, et al. Mitochondrial Correction: A New Therapeutic Paradigm for Cancer and Degenerative Diseases. J Orthomol Med. 2018; 33(4):1-5.
24. Bruno S, Collino F, Deregibus MC, Grange C, Tetta C, Camussi G. Microvesicles Derived from Human Bone Marrow Mesenchymal Stem Cells Inhibit Tumor Growth. Stem Cells and Develop. 2013;22(5): 758-771.
25. Biava PM, Nicolini A, Ferrari P, Carpi A, Sell S. A systemic approach to cancer treatment: tumor cell reprogramming focused on endocrine-related cancers. Bentham Science. 2014;21(9):1072-1081.
26. Livraghi T, Biava PM, Meloni MF, Lazzaroni S, Frati L. Treatment with stem cell differentiation stage factors of intermediate-advanced hepatocellular carcinoma: An open randomized clinical trial. Oncol Res. 2005;15(8):399-408.
27. Fong CY, Chak LL, Biswas A, Tan JH, Gauthaman K, Chan WK, et al. Human Wharton's Jelly Stem Cells Have Unique Transcriptome Profiles Compared to Human Embryonic Stem Cells and Other Mesenchymal Stem Cells. Stem Cell Rev and Rep.2011; (1):1-16.
28. Ayuzawa R, Doi C, Rachakatla RS, Pyle MM, Maurya DK, Troyer D, et al. Naïve human umbilical cord matrix derived stem cells

significantly attenuate growth of human breast cancer cells in vitro and in vivo. Cancer Lett. 2009;280(1):31-37.

29. Gauthaman K, Yee FC, Cheyyatraivendran S, Biswas A, Choolani M, Bongso A. Umbilical cord Wharton's jelly stem cell (hWJSC) extracts inhibit cancer cell growth in vitro. J Cell Biochem. 2012;113(6): 2027-2039.
30. Einhorn L. Are there factors preventing cancer development during embryonic life? Oncodev Biol Med. 1983;4(3):219-229.
31. Biava PM, Canaider S, Facchin F, Bianconi E, Ljungberg L, Rotilio D, et al. Stem Cell Differentiation Stage Factors from Zebrafish Embryo: A Novel Strategy to Modulate the Fate of Normal and Pathological Human (Stem) Cells. Curr Pharm Biotechnol. 2015;16(9):782-792.
32. Proietti S, Cucina A, Pensotti A, Biava PM, Minini M, Monti N, et al. Active Fraction from Embryo Fish Extracts Induces Reversion of the Malignant Invasive Phenotype in Breast Cancer through Down-Regulation of TCTP and Modulation of E-cadherin/Beta-catenin Pathway. Int J Mol Sci. 2019;20(9):2151.
33. Cucina A, Biava PM, D'Anselmi F, Coluccia P, Conti F, di Clemente Ret al. Zebrafish embryo proteins induce apoptosis in human colon cancer cells. Apoptosis. 2006;11(9):1617-1628.
34. Biava PM, Carluccio A. Activation of anti-oncogene p53 produced by embryonic extracts in vitro tumor cells. J Tum Marker Oncol. 1977, 12(4):9-15.
35. Biava PM, Bonsignorio D, Hoxa M, Impagliazzo M, Facco R, Ielapi T, et al. Post translational modification of the retinoblastoma protein (pRb) induced by *in vitro* administration of Zebrafish embryonic extracts on human kidney adenocarcinoma cell line. J Tumor Marker Oncol. 2002;17(2):59-64.
36. D'Anselmi F. Cucina A, Biava PM, Proietti S, Coluccia P, Frati L, et al. Zebrafish stem cell differentiation stage factors suppress Bcl-X release and enhance 5-Fumediated apoptosis in colon cancer cells. Curr. Pharm Biotechnology. 2011;12(2):261-267.
37. Biava PM, Burigana F, Germano R, Kurian P, Verzegnassi C, Vitiello G. Stem Cell Differentiation Stage Factors and their role in triggering symmetry breaking process during cancer development: a quantum field theory model for reprogramming cancer cells to heathy phenotypes. Curr Medi Chem. 2018; 25:1-14.