Exosome Is an Important Novel Way of Acupuncture Information Transmission

Ning-Cen Li, Ming-Yue Li, Zhong-Xi Lu, Mu-Yang Li, Xue-Mao Zhuo, Yong Chen, Ting-Ting Wang, Li-Ying Xing, Mei-Juan Wang, Kai Shan, Ze-Lin Chen, Calista Hui-Min Lim, Bo Chen, Yi Guo

Abstract

As a physical stimulation of the body surface, the process of transmitting acupuncture information from acupoints to target organs through meridians and collaterals is complex, and network regulation is the most basic mode of acupuncture. Exosomes are membrane vesicles formed by various types of cells and secreted to the extracellular matrix through a series of regulation. We speculate that exosomes and their carriers, as important carriers of communication among neurons, endocrine cells, and immune cells, may play an important role in the process of acupuncture information transmission. Exosome in the acupoint area is an important interactive form of transformation from physical information to chemical information. Circulating exosome is an important means of delivery for acupuncture to play an important role in the network regulation. The structure and information molecules of exosomes are the important material bases for acupuncture effect. Through experimental research, it was found that acupuncture can regulate the release of exosome and may have a certain relationship with acupuncture effect. Therefore, exosome may play an important role in the process of acupuncture information transmission, which is an important new way of acupuncture information transmission.

Keywords: Acupuncture, exosome, information transmission, network regulation

Introduction

The initiation process of acupuncture can be divided into three parts: “sensation,” “transmission,” and “effect.” Previous studies have focused on the correlation between acupuncture sensation and efficacy, that is, “Qi Zhi,” and “effective.”[1] However, the process of “transmission” from “sensation” to “effect” is complex. According to the theory of traditional Chinese medicine, it is mainly conducted through the meridians and collaterals. Based on the neurobiological perspective, modern research has made some achievements, but other conduction pathways have not been further studied. How acupuncture physical information is transformed into biological information has not been explained clearly. Prof. Guo Yi’s team focused on the complex process of acupuncture information transmission, which is believed to be closely related to the nerve–endocrine–immune (NEI) network. The team’s latest research found that exosome carriers contain various signal molecules such as neurotransmitters, hormones,
and cytokines. They are carriers that link the relationship between NEI networks and play an important role in the information transmission of acupuncture.

Exosome is a kind of membranous vesicle with a diameter of about 30–200 nm released by the multivesicular bodies. In 2013, it was awarded the Nobel Prize in Physiology or Medicine for its important role in intercellular transport. It can participate in many neural, endocrine, and immune processes such as organ and reproductive development, antigen presentation, immune response, neuron communication, control of aging, and cell proliferation. Exosomes are phospholipid bilayer structures that contain molecules from the source cells, including proteins, nucleic acids, and lipids. Formation of exosomes and their action on target cells are as follows: (1) ligands on exosomes bind to receptors on the receptor cell membrane; (2) exosomes transfer ligands to recipient cells; (3) exosomes themselves fuse with the cytoplasmic membrane to release the “cargo” into the target cell; and (4) endocytosis.

Exosomes have many advantages in the process of intercellular transport: (1) Exosomes from different cells have different functions and carry a variety of substances. They can carry and transfer a variety of biological molecules (proteins, nucleic acids, lipids, etc.) to achieve information exchange (diversity). (2) Exosomes in vitro have a phospholipid bilayer structure of the cell membrane wrapped, which makes the substances transported by exosomes in the process of long-distance transportation not easily degraded like hormones, and the half-life is longer (stability). (3) Exosomes are nanoscale vesicles, which can effectively cross the blood–brain barrier without causing immune and inflammatory reactions (penetration and safety). (4) Because of specific integrin on the membrane surface, exosomes have the characteristics of organ-specific distribution (targetability).

Combined with the characteristics of exosomes, we speculate that exosomes may also play a key role in the transmission of acupuncture information and are important carriers of acupuncture information transmission. After acupuncture, the relevant cells activate and release the exosomes outside. The exosomes in the acupoints transform the physical information of acupuncture into chemical information, activating the initial small network of acupoints, uploading the information to the brain center for integration, and further activating the NEI network. Then, circulating exosomes target delivers the information to the lesion area through the humoral and neural regulation, so as to exert the acupuncture effect.

Exosomes are used as a breakthrough point to study the mechanism of acupuncture information transmission and further regulate the acupuncture effect through the method of artificial intervention of exosomes and their carrier substances, which can promote the maximization of clinical effect, improve the application level of acupuncture and moxibustion, achieve the targeted treatment of diseases, and provide new ideas and approaches for the translational medicine of traditional Chinese medicine with broad application prospects.

EXOSOMES IN THE ACUPUNCTURE AREA ARE AN IMPORTANT INTERACTIVE FORM FOR THE TRANSFORMATION OF PHYSICAL INFORMATION INTO CHEMICAL INFORMATION OF ACUPUNCTURE

Sorting out and analyzing the current domestic and foreign researches related to acupuncture point initiation, it was found that the relevant factors may be related to exosomes. The team further carried out the experimental research and found that exosomes in the acupoint area may participate in the initiation of acupuncture point and regulating exosomes in the acupoint area can significantly affect the acupuncture effect. It was preliminarily confirmed that acupuncture analgesia effect decreased significantly after blocking local exosomes in acupoints.

Acupuncture can lead to tissue winding, pulling, and even fracture in acupoints, resulting in local inflammatory response. Damage-associated molecular patterns activate local cellular functions, and the release of exosomes may be a key link in the conversion of physical information into chemical information by acupuncture.

Acupoint is an important gateway for the interaction of viscera and body surface information. Prof. Guo Yi’s team proposed that it is a sensor and amplifier for acupuncture information. Acupuncture acts on acupoints, first activating the small network of acupoints. The information of acupuncture is amplified by cascade, and the NEI network is activated and further outputs the effect information to the target organ through multilevel and multi-systems and finally acts on the disease network to produce acupuncture effect. Realizing “three-network linkage” of acupoint network, NEI network, and target organ pathological network, acupoints are the “signal source” in the process of acupuncture network.

Acupuncture is a kind of microtraumatic physical stimulation. During the process of acupuncture, the pulling and squeezing of the subacupoint connective tissue network scaffold are caused by lifting, inserting, and twisting, and the deformation and displacement of the associated tissue are caused by the winding of needle body and connective tissue. For example, damage-associated molecular patterns (DAMPs) can be released when the needle-centered spiral pattern is observed in the tissues of the acupoint area, and the muscle tissue in the acupoints after acupuncture has muscle fiber winding, pulling, and even fracture, and there is inflammatory cell infiltration in the gap. DAMPs can activate the function of local cells in acupoints and release exosomes. The chemical substances act on the receptors or ion channels related to nerve endings directly or through the exosomes to stimulate the nerves, and the physical stimulation of acupuncture can be transformed into chemical information. It is found that in the absence of pathogenic diseases, local tissue damage can lead to the upregulation of exosome-related DAMPs, and these molecules play an important role in further reaching various tissues of the body through exosomes circulation. For example, exosomes derived from keratinocytes of the skin contain heat shock
protein 70, a damage factor, which has a protective effect on neurons when they are damaged.[11]

In the early stage, we used electroacupuncture to treat adjuvant arthritis rats as the effect platform. By injecting exosomes antagonists – GW4869 into the acupoint, it is found that regulating the exosomes in acupoints can affect the acupuncture effect. It was preliminarily confirmed that the acupuncture analgesia effect was significantly weakened after blocking the local exosomes of the acupoints, indicating that the local exosomes of the acupoints may participate in the starting process of acupuncture acupoint effect.[12]

**Acupuncture can activate the degranulation of mast cells in acupoints and transport many chemical substances through exosomes, further acting on other cells and tissues**

In acupuncture treatment, acupoint specificity is regarded as a potential principle of acupoint selection. According to the theory of traditional Chinese medicine, stimulating acupoints can cause functional response so as to achieve the purpose of treating diseases. The shape and size of exosomes are not specific. They are cup-shaped structures measuring 40–200 nm under transmission electron microscope. Exosomes are formed by endosome system and have cell membrane structure. The functions of exosomes secreted by different cells are due to the different substances carried by exosomes, including DNA, RNA, proteins, and lipids, which also provide a theoretical basis for the participation of exosomes in acupoint specificity. In the case of mast cells, mast cells are the cells existing in the human body, which have more compounds than other similar functional cells. Mast cells are one of the most profound functional cells in the study of cellular function, meridian response, and acupuncture effect. Some researchers used histomorphology, cell biology, molecular biology, and other technologies to study the degranulation and acupoint specificity of mast cells in the acupoint area. The results showed that there was a high density of mast cells in the acupoint tissue compared with nonacupoint area, with obvious acupoint-specific distribution. Degranulation of the mast cells is one of the signals of acupuncture points.[13]

A new study in 2019 showed that mast cell-derived exosome (Mc-exo) contains up to 1988 proteins, 397 IncRNAs, and 272 microRNAs, and the diversity of information it carries lays the biological foundation for elucidating the function of Mc-exo.[14] Combined with the structure and function of mast cells, for the first time, we proposed an important role of Mc-exo in the initiation and conduction of acupuncture acupoints – acupuncture can cause the enrichment of mast cells and promote the degranulation of local acupoints. Many chemicals are released in the form of microvesicles or exosomes, which play a corresponding role in other cells and tissues.[15] The existence of “synaptic connections” between mast cells and nerve endings provide an anatomical basis for exosomes to interact with each other. It is found that MC-exo can mediate the activation of B-lymphocytes and T-lymphocytes and activate the immunoregulation. It can also activate the TRPV receptor at sensory nerve endings and initiate nerve regulation so that the acupuncture effect can be continuously amplified in acupoints.[16]

**Acupuncture activates local nerve fibers through acupoint receptors to initiate nerve regulation. Exosomes are an important mode of communication between cells and sensory nerve endings**

Exactly, the pathway of action of exosomes is not directly related to membrane potential, that is, to say, the release of exosomes will not directly cause nerve action potential. As a new way of intercellular information transmission, exosomes are an important mode of communication between cells and sensory nerve endings. The release of exosomes with presynaptic neurotransmitters can enhance postsynaptic action potential and synaptic connection strength. After acupuncture, local nerve fibers are activated by receptors, and then, more nerve fibers are activated by local axonal reflex. Neuronal synapse excitation can release exosomes, which are received by other designated neurons, realize the communication and transmission of information between nerve cells, and initiate nerve regulation. Peripheral nerve fibers can also express the substance P and calcitonin gene-related peptide, which act on the local blood vessels, further increase blood flow, exudate plasma, aggregate and degranulate mast cells, release exosomes that contain pain causing and sensitizing substances such as tryptase, 5-HT, HA and so on, and jointly complete the local histochemical reaction process of acupoints.[17]

To sum up, acupuncture can cause changes in the local microenvironment of acupoints, including activation of cell functions, release of chemical substances, and excitation of afferent nerves. The interaction between these factors may be realized through exosomes, which together constitute an important biological basis for information initiation of local acupuncture effect at acupoints.

**Circulating Exosomes are an Important Transport Method for Acupuncture to Play its Role in Network Regulation**

**Neuro–endocrine–immune network is the biological basis for human body to maintain homeostasis and an important link for the regulation of acupuncture network**

Modern research shows that organism is a complex system formed by the interaction of numerous networks. There is a network “universal language” among the three systems of nerve, endocrine, and immune, which is closely linked. The connection among the three is the two-way regulation between the two, while any change of one system will directly or indirectly affect other systems, thus forming the body’s stable regulation system “NEI network.”[18]

A study by the Mayo Clinic, one of the world’s most influential medical institutions and representative of the world’s strongest medical standards, points out that the complex mechanism
of acupuncture cannot be explained by a single mechanism. Acupuncture treatment of diseases is achieved by stimulating the body’s overall network regulation. From acupoint stimulation to the body effect, it is not a simple direct linear connection but mediated by the complex network regulation system in vivo, namely NEI network. A large amount of experimental studies showed that the needle acupuncture effect is closely related to the three systems; Prof. Guo Yi’s team took mast cells as the breakthrough point for research in the early stage and found that not only mast cells are related to the production of acupuncture effect, but also mast cell degranulation can further activate neural, endocrine, and immune network so as to cascade amplification to play acupuncture effect.\(^{[19,20]}\)

Further studies have found that acupuncture at Zusanli (ST36) not only improves the immune and humoral functions of rats but also increases neurotransmitters such as substance P and vasoactive intestinal peptide. Some common mediators of NEI network, such as interleukin-6 (IL-6), IL-6R, endorphin, corticosteroid-releasing hormone, adrenal cortex hormone, and corticosterone, were found to be involved in the regulation of electroacupuncture in the treatment of aging rat models.\(^{[21]}\) However, most of these studies only take some indicators in the “universal language” as the observation object and lack of overall grasp and systematic study of NEI network, which still have some deficiencies.

**As an important carrier of the intercellular communication among nerve, endocrine, and immune cells, circulating exosomes are an important transport method for acupuncture to play its role in network regulation**

Circulating exosomes and their carriers are important carriers of intercellular communication such as NEI. They can participate in various aspects of the development and function of the nervous system and further regulate endocrine and immune responses through intercellular transport and exchange of substances. For example, electroacupuncture at Quehi (LI11) and Zusanli (ST36) points can promote the secretion of exosomes by M2 microglia in the motor cortex of the ischemic side of rats, play a neuroprotective role, and improve motor function.\(^{[22]}\) Electroacupuncture can change the expression profile of circRNA of the plasma exosomes in type 2 diabetic mice, and it has an intervention effect through metabolism, cell growth, development, and other functions.\(^{[23]}\) Electroacupuncture can upregulate the expression of miR-181 in the serum exosomes and increase renal blood flow.\(^{[24]}\) Some scholars believe that exosomes may be related to meridian activities, and the theory of viscera in traditional Chinese medicine may predict the route of exosomes.\(^{[25]}\)

We found that electroacupuncture can promote the release of serum exosomes in normal rats. Within a certain range, the expression of exosomes can increase with the increase of acupuncture times. For adjuvant arthritis rats, electroacupuncture can inhibit the pro-inflammatory circulating exosomes in the early stage and release the anti-inflammatory exosomes in the later stage, so as to play an analgesic and anti-inflammatory role.\(^{[26]}\) Further, by intraperitoneal injection of normal rats circulating exosomes, acupuncture normal/model rats circulating exosomes, and exosomes antagonist GW4869, it was found that the pain threshold of rats was significantly increased on the 1\(^{st}\) day after injection of acupuncture normal rats, as the analgesic effect of acupuncture was simulated. Similar effect appeared on the 3\(^{rd}\) day after injection of acupuncture model rats circulating exosomes. However, injection of the normal rats circulating exosomes without acupuncture had no effect on the analgesic effect, and the analgesic effect of acupuncture after injection of antagonists was significantly weakened. The NEI system is an interconnected whole, and the overall regulatory effect of acupuncture is realized through this network. Exosomes are an important transport method for the regulation of acupuncture network.

**EXOSOMES STRUCTURE AND CARRYING INFORMATION MOLECULES ARE THE IMPORTANT MATERIAL BASES OF ACUPUNCTURE-TARGETING EFFECT**

Acupuncture at meridians and acupoints has the characteristics of guiding drugs to the lesion sites “Qi to the place of disease” is also known as “Xing Qi.” It refers to the phenomenon that the doctor after getting Qi uses certain techniques to make the acupuncture sense transmit to the affected area along the meridian. Qi to the bottom of the needle is the starting point, while Qi to the place of disease is the end point. The so-called “Qi” refers to the feeling of acid, numbness, heaviness, and distention after acupuncture, that is, acupuncture induction, “acupuncture sensation.” The therapeutic effect of acupuncture is closely related to the degree of sensorineural transmission. Ancient doctors have taken stimulating sensorineural transmission and promoting Qi as positive means to improve the therapeutic effect of acupuncture. Modern studies have found that acupuncture can guide tumor chemotherapy drugs and gather to lesions. For example, paclitaxel concentration in the tumor body significantly increased and induced tumor cell apoptosis after electroacupuncture encircles breast cancer mice, which may be achieved by regulating tumor microvascular and microenvironment to promote tumor local drug concentration.\(^{[27]}\) Acupuncture at “Feishu” (BL13) and “Lingtai”(DU10) could affect the distribution and metabolism of paclitaxel in the tissues of lung cancer mice and the drug content targeting to the lungs increased, while the drug content in the spleen and kidney decreased, suggesting that “acupuncture and drugs work together” could prolong the stay time of the drug in the tissues and play a synergistic role.\(^{[28]}\) Acupuncture at the meridians and acupoints related to the viscera can arouse the sensation conduction along meridian to the disease site and further improve the curative effect.
The structure and surface-specific proteins of exosomes are the important material basis for their targeting

Exosomes, as a new way of intercellular communication, have been used as the delivery medium to deliver various effective bioactive substances to the recipient cells. According to the latest statistics of Vesiclepedia database (http://www.microvesicles.org), 349,988 kinds of proteins, 27,646 kinds of mRNAs, 10,520 kinds of microRNAs, and 639 kinds of lipids have been identified in exosomes, which provide a basis for the multi biological activity of exosomes. At present, there are some problems in the drug transport carrier, such as short transport time, poor stability, easy to be cleared by the body, and low targeting. However, exosomes are released from the cells of the body itself, which can significantly reduce immunogenicity and toxicity and improve biocompatibility. The specific integrins on the surface make the exosomes preferentially fuse with the cells in the target organs or tissues so as to achieve the targeted treatment of diseases. Some researchers attached hydrophobic curcumin to the outer membrane of exosome and concentrated exosomes with curcumin before administration. It was found that the drug concentration and administration efficiency of curcumin could be greatly increased.[29]

Studies have found that integrins, extracellular matrix (ECM) proteins, lectins, proteoglycan, and glycolipids on the exosomes play an important role in the process of targeting to specific receptor cells. For example, exosomes expressing ITGα6β4 and α6β1 may lead to lung metastasis, while those expressing ITGα5β5 will cause liver metastasis. [30] When exosomes are directed to the liver by αVβ5, they also cause the synthesis of the damage-related factor S100 protein, which promotes inflammation and cell migration.[31] After antagonizing ITGα6β4 and αVβ5, the absorption of exosomes by target cells was reduced, and the probability of cancer cells transferring to lung and liver was also reduced. Further injection of lung cancer exosomes into pretreated mice could lead bone metastasis tumor cells back to lung. Some researchers further injected 125I fluorescently labeled B16BL6 exosomes intravenously in mice and found that they were widely concentrated in the liver and increased with time, reaching a peak at 30 min.[32,33] Other scholars have found that breast cancer cells MDA-MB-231-derived exosomes (231-exo) are preferentially located in the lung, which is mediated by the high binding affinity between lung epithelial cells and 231-exo surface integrin ITGβ4.[34]

Exosomes may be the important material basis for the acupuncture targeting effect

It was found that different types of integrins secreted by different cells can target different organs, and the exosomes can also change their selectivity by affecting the premetastasis microenvironment of specific organs. For example, tumor-derived exosomes can carry cytokines such as transforming growth factor-beta, caveolin-1, HIF1α, and β-catenin to improve the ability of invasion and metastasis of receptor cells, differentiate fibroblasts and mesenchymal cells, remodel extracellular matrix, induce epithelial mesenchymal transformation and angiogenesis, and change the local microenvironment to accelerate tumor growth and metastasis. Integrins on tumor-derived exosomes are preferentially absorbed by cells close to organs.[35]

Prof. Guo Yi’s team took acupuncture treatment of adjuvant arthritis rats as the research platform, using small animal in vivo imaging technology to find that acupuncture biomass not only entered the blood circulation but also entered the cerebrospinal fluid circulation and also reached the brain through the blood–brain barrier, but the way is not clear. It is speculated that it may be realized by exosomes carrying through the blood–brain barrier. We used label-free quantitative method to analyze the protein types and contents in the serum exosomes of rats with adjuvant arthritis treated by electroacupuncture. It was found that the expression of these proteins was increased in the plasma, inflammatory cells, synovial fluid, cerebrospinal fluid, glandular tissue, and muscle. The increase of expression in the lesion may be related to the targeting characteristics of exosomes, suggesting that exosomes can change the local microenvironment of the joint. To meet the demand of the local lesions, the effective components of acupuncture can move in a targeted way, i.e., “Qi to the place of disease.”

Summary

Exosomes and their carriers are the important material bases and may play a key role in the process of acupuncture multichannel network regulation. After acupuncturing, the local fibrous tissue is wound, broken, and damaged; DAMPs are released, further activate the corresponding cells, release exosomes, and activate the initial small network of acupoints. The chemicals directly or through the exosomes are carried to the related receptors or ion channels of the synapses of nerve endings and then make nerve excitement. Acupuncture information is uploaded to the central nervous system to integration through the spinal cord and down to regulate the important organs or tissues to activate the NEI network. Exosomes further target information to the lesion site through the regulation of body fluids and nerves, thus exerting the acupuncture effect. Exosomes and their transporters, as the important carriers of communication among neuro, endocrine, and immune cells, may be the key material basis in the complex network process of signal initiation, conduction, and targeting of acupuncture signals to the lesion sites [Figure 1].

Deficiency and Prospects

As a kind of physical stimulation therapy, although some studies have been carried out on the onset process of acupuncture from the perspective of biochemistry, most of them focus on the changes of related biochemical substances and pay less attention to how the physical information of acupuncture transforms into chemical information, so it is difficult to explain the universal mechanism of acupuncture onset. Although we suggest that exosomes may play an
important role in the transformation of acupuncture from physical information to chemical information, how to study it is still a black box. How to observe the exosomes in acupoints intuitively, how to trace the source of circulating exosomes (from which cells or tissues), and which lesions are targeted after the release of circulating exosomes still need to be studied. All of these require more advanced technology to solve.

Ethical Statement
Our experiments in this study were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal care and experimental procedures used in the current study were approved by the Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (Permit Number: TCM-LAEC2014015). All efforts were made to minimize suffering of animals.

Authorship contributions
Li Ningcen, Li Mingyue, Lyu Zhongxi, Chen Bo and Guo Yi wrote this paper, while Chen Bo and Guo Yi were co-corresponding authors. Li Ningcen, Li Muyang, Zhuo Xuemao, Chen Yong, Wang Tingting and Xing Liying carried out the experiments. Wang Meijuan and Gong Yinan translated this paper. Chen Zelin has checked the whole manuscript. All authors made substantial contributions to discussion of content and to review and edit the manuscript before submission.

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Conflicts of interest
There are no conflicts of interest.

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