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SPECIAL ARTICLE

Simulations of centriole of polarized centrosome as a monopole antenna in immune and viral synapses

Josef Dvorak[1], Bohuslav Melichar[2], Alzbeta Filipova[3], Jana Grimova[4], Nela Grimova[5], Aneta Rozsypalova[1], David Buka[6], Rene Voboril[7], Radek Zapletal[1], Tomas Buchler[1], Igor Richter[8]

[1]Department of Oncology, First Faculty of Medicine, Charles University and Thomayer Hospital, Prague, Czech Republic; [2]Department of Oncology, Palacky University Medical School and Teaching Hospital, Olomouc, Czech Republic; [3]Department of Radiobiology, Faculty of Military Health Sciences in Hradec Kralove, University of Defense in Brno, Hradec Kralove, Czech Republic; [4]Department of Oncology, University Hospital in Motol, Prague, Czech Republic; [5]Faculty of Electrical Engineering, Czech Technical University, Prague, Czech Republic; [6]Department of Oncology and Radiotherapy, Charles University, Medical School and Teaching Hospital, Hradec Kralove, Czech Republic; [7]Department of Surgery, First Faculty of Medicine, Charles University in Prague and Na Bulovce Hospital, Prague, Czech Republic; [8]Department of Oncology, Regional Hospital, Liberec, Czech Republic

Summary

The immune synapse (IS) is a temporary interface between an antigen-presenting cell and an effector lymphocyte. Viral synapse is a molecularly organized cellular junction that is structurally similar to the IS. Primary cilium is considered as a functional homologue of the IS due to the morphological and functional similarities in architecture between both micotubule structures.

It has been hypothesized that endogenous electromagnetic field in the cell is generated by a unique cooperating system between mitochondria and microtubules. We are extending this prior hypothesis of the endogenous electromagnetic field in the cell postulating that polarized centriole in immune and viral synapse could serve as a monopole antenna. This is an addition to our hypothesis that primary cilium could serve as a monopole antenna.

We simulated the distribution of electric field of centriole of polarized centrosome as a monopole antenna in immune and viral synapse. Very weak electromagnetic field of polarized centriole of CD8+ T lymphocyte in IS can contribute to the transport of cytolytic granules into the attacked (cancer) cell. Analogically, very weak electromagnetic field of polarized centriole in viral synapse of infected CD4 cells can aid the transport of viruses (human immunodeficiency virus) to non-infected CD4 cells.

We hypothesized that healthy organisms need these monopole antennas. If, during the neoplastic transformation, healthy cells lose monopole antennas in form of primary cilia, the IS aims to replace them by monopole antennas of polarized centrioles in IS to restore homeostasis.

Key words: *distribution of electric field models, immune synapse, monopole antennas, primary cilia, viral synapse*

Introduction

The IS is the temporary interface between an antigen-presenting cell and an effector lymphocyte. IS is also known as the supramolecular activation cluster (SMAC). This structure is composed of concentric rings, each containing segregated clusters of proteins, including central-SMAC, peripheral-SMAC and distal-SMAC. This complex as a whole has several functions, encompassing

Correspondence to: Josef Dvorak, MD, PhD. Department of Oncology, First Faculty of Medicine, Charles University and Thomayer Hospital, Videnska 800, 140 59 Prague, Czech Republic.
Tel: +420 261083492, Fax: +420 261082522, E-mail: josef.dvorak@ftn.cz
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regulation of lymphocyte activation, transfer of peptide major histocompatibility complexes from antigen presenting cells to lymphocytes and direct secretion of cytokines or cytolytic granules. The IS integrates three broad categories of receptors, i.e. antigen T cell receptor, adhesion, and costimulatory/checkpoint [1]. Key molecules in the synapse are the T cell receptor and its counterpart the major histocompatibility complex.

A key event in IS-induced cell polarization is movement of the centrosome right up to the membrane at the edge of the central-SMAC, initially reported in cytotoxic T lymphocytes and more recently in CD4+ T helper cells as well as natural killer (NK) and natural killer T (NKT) cells. As the centrosome is the only microtubule organizing centre (MTOC) of cytotoxic T lymphocytes, its movement induces reorganization of the intracellular microtubule cytoskeleton, which is thought to allow polarized secretion of cytolytic granules at the IS [8]. Cytotoxic T lymphocytes, CD4+ T helper cells, NK and NKT cells employ the centrosome for organization of microtubules' minus-ends at the point of signalling on the plasma membrane. The minus-end directed movement of cytolytic granules allows precise delivery to the IS [2].

Viral synapse is a molecularly organized cellular junction that is similar in some aspects to the ISs. Viruses use the microtubule cytoskeleton to migrate to the viral synapse. Many viruses, including herpes simplex virus (HSV), human immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV) have been shown to instigate the formation of these junctions between the infected ("donor") and uninfected ("target") cell to allow cell-to-cell transmission. Viral synapses are thought to explain how cell-to-cell transfer can operate in the HIV infection even when there is a low number of viral particles and a relatively low number of CD4+ T helper cells receptors [3]. These findings highlight a previously unappreciated role for the viral synapse in the HIV pathogenesis. As viral synapses allow the virus to spread directly from cell to cell, they also provide a means by which the virus can escape neutralizing antibodies.

The primary cilium is an immotile solitary sensory microtubule-based organelle protruding from the surface of the majority of human cells. As post-mitotic cellular structures primary cilia are present during G_0/G_1 and the outset of S phase. Primary cilia disassemble at late S phase or start of G_2 when centrioles, including the centriole functioning as a basal body, are required to organize the mitotic spindle, and resorption of primary cilium is essential for cell cycle re-entry [4].

Primary cilium is considered as a functional homologue of the IS due to the morphological and functional similarities in architecture between both micotubule structures. In both cases endocytosis and exocytosis are focused at the point of centrosome docking [5], ciliary intraflagellar transport and Hedgehog proteins are found in T cells, and both structures form important signaling platforms [2,6]. This highlights a possible origin of the IS as a modified cilium [6].

Endogenous electromagnetic field in the cell: Published hypotheses about the mechanisms of its generation

It is hypothesized that the endogenous electromagnetic field (EMF) in the cell is generated by a unique cooperating system between mitochondria and microtubules, as has been reviewed previously [7]. Besides the production of adenosine (ATP) and guanosine triphosphate (GTP) mitochondria form a proton space charge layer, strong static electric field, and water ordering around them in cytosol - that represent the necessary conditions for generation of coherent electrodynamic field by the microtubules. Electrodynamic forces are of a long-range nature in comparison with the bond and cohesive forces [8].

We are adding to the existing hypothesis of the endogenous EMF in the cell, with which we agree, the present hypothesis, that primary cilium (Figure 1) and polarized centriole in the immune and viral synapse could serve as a monopole antenna (Figures 2 and 3).

Hypothetical model of centriole of polarized centrosome as a monopole antenna in the immune and viral synapse

Centrioles are 0.25 μm in diameter and their length may vary according to the species, around 0.4-0.5 μm. We hypothesize that polarized centriole in the region of immune and viral synapse could serve also as monopole antenna. This is an extension of our hypothesis, that primary cilium could serve as a monopole antenna. The length of centriole of polarized centrosome (monopole antenna) could predict the wavelength (inverse of frequency) of radiated EMF in water environment. However, the emitted and received wavelength could be longer than the centriole. For example in the technical applications, the most common is the quarter-wave monopole antenna in which the antenna is approximately 1/4 of a wavelength of the electromagnetic waves [9].

In contrast with the primary cilium for which the length of the monopole antenna is increasing

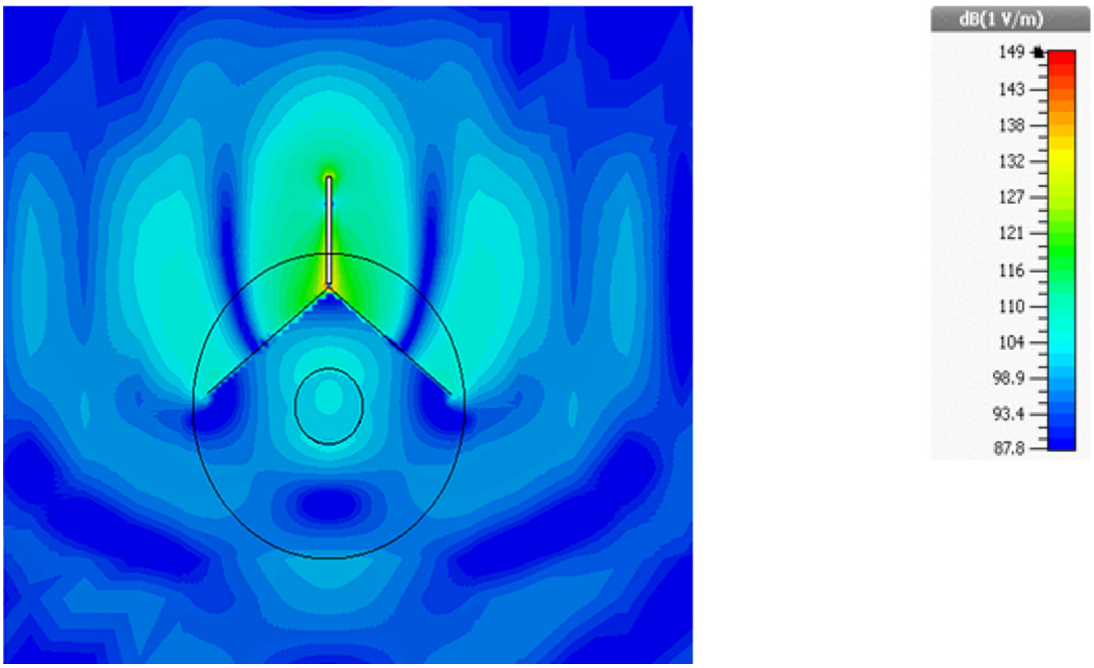

Figure 1. Contour plot of the distribution of electric field of endogenous EMF of primary cilium of cancer-associated fibroblast as a monopole antenna. The simulation was performed using software computer simulation technology AG, Darmstadt, Germany (CST).

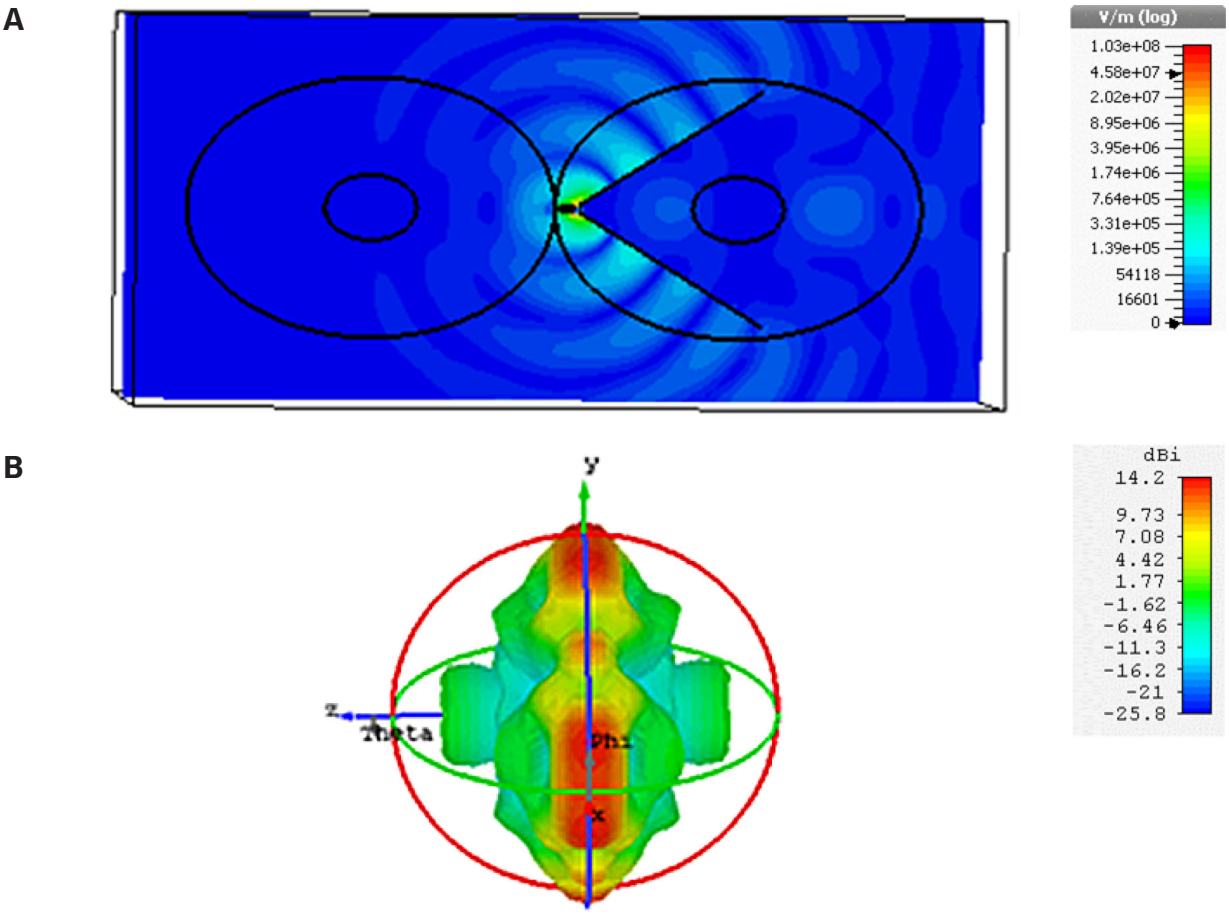

Figure 2. A: Contour plot of the distribution of electric field of the polarized centriole (as a monopole antenna) of cytotoxic CD8+ TIL in the region of immune synapse: left: cancer cell and right: cytotoxic CD8+ TIL. Cytotoxic CD8+ TIL, when forms immune synapse, polarizes centriole unlike attacked cell, which does not polarize centriole. **B:** Radiation pattern of endogenous EMF in the region of the contact between effector and antigen-presenting cell. The simulations were performed using software computer simulation technology AG, Darmstadt, Germany (CST).

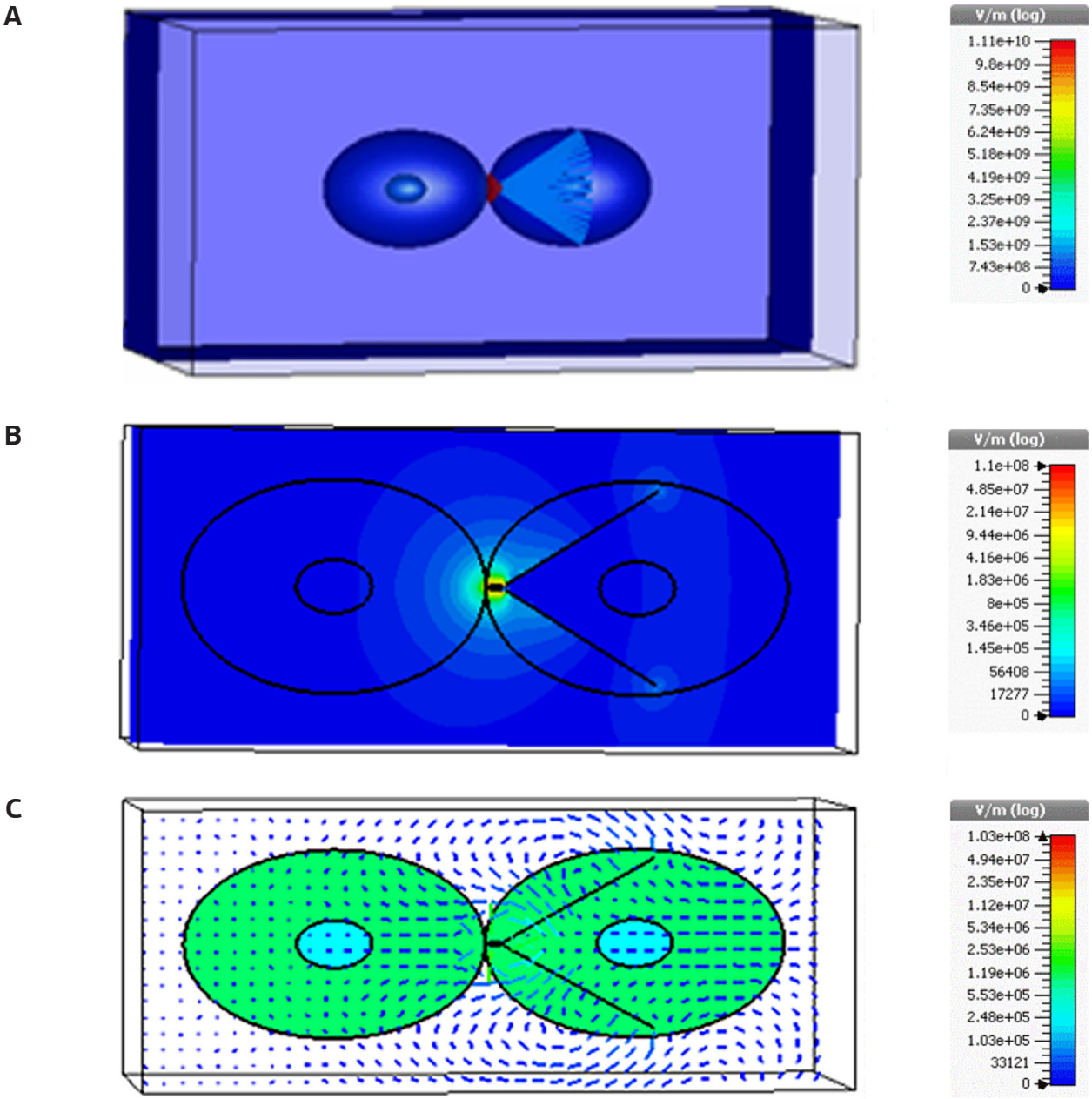

Figure 3. A: Model of the polarized centriole (as a monopole antenna) of the infected CD4+ T helper cell in the region of viral synapse: left: attacked normal CD4+ T helper cell and right: HIV infected CD4+ T helper cell. Infected CD4+ T helper cell which form viral synapse polarizes centrosome in contrast to attacked normal CD4+ T helper cell that does not polarize centrosome. **B:** Contour plot of the distribution of electric field of the polarized centriole of the infected CD4+ T helper cell in the region of viral synapse: left: attacked normal CD4+ T helper cell and right: infected CD4+ T helper cell. **C:** Arrow plot of the distribution of electric field of the polarized centriole of the infected CD4+ helper cell in the region of viral synapse: left: attacked normal CD4+ helper cell and right: infected CD4+ helper cell. The simulations were performed using software computer simulation technology AG, Darmstadt, Germany (CST).

during the quiescent phase of the cell cycle from the length of centriole to several microns (proportionally is increasing the emitted and received wavelength and inversely decreasing the frequency), the length of monopole antenna of polarized centriole in the immune and viral synapse is always the same. Another difference is the period of radiation. The duration of the quiecent phase of the cell cycle with formed primary cilium in nonhematopoietic cells can take many days, but the duration of polarized centriole in immune and viral synapse is much shorter. For example, cytotoxic T lymphocytes can kill targets within 5 min of interaction while moving between multiple targets [10], therefore the time of centrosome interaction with the plasma membrane must be brief [11].

Normal, nonhematopoietic cells form primary cilium which could serve as a monopole antenna.

The loss of primary cilium (monopole antenna) is an early event in the neoplastic transformation. The majority of cancer cells lack the primary cilia, or possess them damaged or at a low frequency [12]. Cancer-associated fibroblasts possess the primary cilia. Recently we reported positive prognostic significance of primary cilia in the tumor microenvironment in intestinal cancer [13].

An association of high density of CD8+ tumor infiltrating lymphocytes (TIL) with favorable prognosis and treatment outcome has been repeatedly demonstrated across a spectrum of different primary tumors, yet not only in primary tumors but also in visceral and brain metastases [14]. In colorectal cancer the density of cytotoxic and memory T lymphocytes in the center and the invasive margin of the tumor is assessed according to Immunoscore. The more the cytotoxic CD8+ TIL in tumor microenvironment present, the better the prognosis of cancer [15].

The final effector mechanism of antitumor immune response relies mainly on cytotoxic T lymphocytes recognizing nonself antigens, ultimately leading to tumor cell killing via the IS [16], including the formation of monopole antenna. The IS is a temporary interface between an antigen-presenting or cancer cell and the effector lymphocyte [1]. One mechanism by which cancer cells limit the formation of the IS is the upregulation of programmed death-1 ligand (PD-L1) and its binding to programmed death protein-1 receptor (PD-1) on CD8+ TIL (termed adaptive immune resistance). Immune checkpoint blockade using anti-PD-1 antibodies, such as nivolumab and pembrolizumab, currently appears to be the most promising approach. This innovative immunotherapy differs from the conventional cancer treatment by the ability to produce durable responses in some patients [17]. It is expected, that in future not only blocking the negative pathway but also agonist drugs, could support the formation of IS between the CD8+ TIL and cancer cell.

Both microtubule structures, i.e. primary cilia of cancer-associated fibroblasts and ISs between cytotoxic CD8+ TIL and antigen-presenting or cancer cells, are regularly encountered at varying frequency in the microenvironment of solid tumors. These could, in fact, represent two sides of the same coin [18]. To investigate solely the CD8+ TIL frequency may provide only a part of the information. The second part of the information may be the frequency of primary cilia in the tumor microenvironment, which is not routinely investigated, though it is not difficult nor expensive to assess.

We hypothesize that healthy organisms physiologically need monopole antennas of primary cilia and polarized lymphocytes. If, during the neoplastic transformation, cells lose monopole antennas of primary cilia, they are replaced by monopole antennas of polarized centrioles in ISs to restore homeostasis.

It is a fundamental property of antennas that the electrical characteristics of an antenna are the same whether the antenna is transmitting or receiving as postulated by the reciprocity theorem of electromagnetics [9]. A necessary condition for this reciprocity property is that the materials in the antenna and transmission medium are linear and reciprocal meaning that the material has the same response to an electric current or magnetic field in one direction, as it has to the field or current in the opposite direction. In all models we assume this reciprocity. The reported values of conductivity of microtubules vary considerably in the literature [19]. We hypothesize that the polarized centriole as monopole antenna can serve for both transmitting and receiving signals at the same frequency.

The overall power production of the living cell, determined with calorimetric measurements, is in the order of magnitude of 0.1 pW [20]. The power of polarized centriole as monopole antenna in the immune and viral synapse has to be lower than this value.

The function of centrioles has long been mysterious and controversial due to the desire to assign a mitotic role to these organelles despite an ever increasing list of experiments showing that mitosis can proceed rather well when centrioles are removed from cells (e.g. by laser ablation) [21,22]. On the other hand, centrioles are absolutely necessary for the formation of primary cilia. There is no observed case of primary cilium in the absence of centrioles. The quiescent phase of cell cycle usually takes much longer than mitosis (less than one hour). In adult organisms, most cells exist in a quiescent state, with the need to respond to transient environmental signals sensed through the primary cilia [23]. Centrioles are also necessary for the formation of immune and viral synapses. We hypothesize that the main function of centrioles is the formation of monopole antennas.

Simulations

All simulations were performed using software Computer Simulation Technology AG, Darmstadt, Germany (CST). All models were simulated in water environment. For the simulation of the propagation of EMF in water the Debye model was used (Figures 1-3). We simplified the structure of microtubules in the shape of a cone in the models.

Discussion

IS plays an important role in the communication between immune cells by focusing signaling, secretion, and endocytosis at the point of contact between effector and antigen-presenting cells. Cytotoxic T lymphocytes use polarized secretion to rapidly destroy virally-infected and neoplastically-transformed cells [24]. Very weak EMF of polarized centriole of CD8+ TIL in the IS can contribute to transport signaling molecules and cytolytic granules into the attacked cell (Figure 2).

In the event of failure of signaling between CD8+ TIL and another cell, a similar mechanism can lead to the destruction of certain healthy cells types in autoimmune diseases such as multiple sclerosis, coeliac or Crohn's disease. It can also contribute to autoimmune side effects of check point inhibitors treatment. Very weak EMF of polarized centriole of IS could participate in these processes.

The radiation of EMF of primary cilium as monopole antenna is terminated by the dissolution of primary cilium at the end of the quiescent phase of the cell cycle. The IS, including the association EMF radiation, is terminated by the destruction of the attacked cell. In an optimal case, cancer cell or viral-infected cell are destroyed, but in case of failure of signaling between cytotoxic CD8+ TIL lymphocyte it may cause an autoimmune destruction of some types of normal cells.

Analogically, the very weak EMF of polarized centriole in viral synapse of infected CD4+ T helper cells can help the transport of viruses (e.g. HIV) to non-infected CD4+ T helper cells (Figure 3). Infected CD4+ T helper cells, which form viral synapse, polarize the centrosome in contrast to attacked CD4+ T helper cells that do not polarize centrosome. Even if the emitted power is low, it may have an impact on intracellular processes such as the transport of substrates or temporal and spatial organization structures and molecules, and may interact with the environment such as the adherence of cells, force acting on the particles of the dielectric at a distance and/or intercellular interactions [25].

According to simulations of the distribution of electric field of polarized centriole as a monopole antenna in immune and viral synapse, the electromagnetic waves radiate not only to the neighbouring cells, but also in the nucleus of the same cell (Figure 2-3), changing the gene expression.

Differentiated enterocytes show permanently polarized centrosomes at the apical surface, but lose the cilia as embryogenesis and tissue differentiation progress [26]. The centrosome remains at the surface associated with a vestigial cilium remnant but the adult tissue entirely lacks mature cilia, suggesting downregulation or loss of cilia mechanisms with the development and differentiation [11]. We hypothesize that polarized centrioles of differentiated enterocytes can also serve as monopole antennas.

The present pilot study provides the first simulations of centriole of polarized centrosome as a monopole antenna in immune and viral synapses.

The potential implications of the function of centriole of polarized centrosome as a monopole antenna in immune and viral synapse

It was recently reported that T lymphocytes possess intrinsic photosensitivity in the blue light region at the wavelength 480 nm and this property may enhance the motility in skin caused by sunlight. Photosensitivity is greater in activated T cells and mediated by the H_2O_2 signaling pathway [27]. This wavelength corresponds to the length of the polarized centriole in immune and viral synapse and short primary cilia. Therefore, we hypothesize that photosensitive functions of T lymphocytes can be influenced not only by sunlight in the skin but also by the centriole of polarized centrosome as a monopole antennas in immune and viral synapse and short primary cilia as monopole antennas in the whole organism. The radiation power is weaker than sunlight, but can effect on shorter distance and in the case of short primary cilia for the relatively long time of the quiescent phase of the cell cycle.

The proof of the function of polarized centriole in immune and viral synapse as monopole antennas could advance our understanding of the nature of life from molecular biology and chemistry deeper in the physics. It could extend our diagnostics and treatment modalities.

Experimental tests for the verification of the function of centriole of polarized centrosome as a monopole antenna in immune and viral synapse

There are several ways how to verify this hypothesis. For example it is possible to use the voltage sensitive dyes (nanosized voltmeter) in the microenvironment outside the immune and viral synapse [28] or indirect cellular EMF detection by micro-dielectrophoresis [29]. During the measurement, the cells must be at room temperature alive and able to communicate. The main technical problem is the very weak radiation power of the primary cilium (less than 0.1 pW) at the wavelength of several microns measured in room temperature background.

It is possible to use photon counting with low noise and highly sensitive photon counting system that is capable of spectral analyses. The system, located in dark room, consists of a light-tight sample chamber, a photomultiplier tube, a rotating wheel with a set of glass filters for spectral analysis and computer-controlled photon counter. Electronic noise in photomultiplier tube can be decreased by water cooler or thermoelectrically.

In the experiments we expect the higher photon emission at the wavelength proportional to the length of polarized centrioles in immune and viral synapses in comparison with the same amount of the same type of cells without polarized centrioles measured in the same conditions.

Conclusion

In summary, we hypothesize that the polarized centriole in the immune and viral synapse can serve as a monopole antenna for both transmitting and receiving signals. The present report provides the first simulations of electromagnetic field of radiation pattern of centriole of polarized centrosome as a monopole antenna in immune and viral synapse in water environment. Very weak electromagnetic field of polarized centriole of CD8+ T lymphocyte in IS can contribute to transport of cytolytic granules into the attacked cell (e.g. cancer cell). Analogically, a very weak electromagnetic field of polarized centriole in viral synapse of infected CD4 cells can aid to transport viruses (e.g. human immunodeficiency virus) to non-infected CD4 cell. We hypothesize that healthy organisms physiologically need monopole antennas of primary cilia and polarized lymphocytes. If, during the neoplastic transformation, cells lose monopole antennas of primary cilia, they are replaced by monopole antennas of polarized centrioles in IS to restore homeostasis. This hypothesis could be verified by voltage-sensitive dyes (nanosized voltmeter) or photon counting.

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Conflict of interests

The authors declare no conflict of interests.

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