Description for the general public

The main goal of this innovative project is to explain and clarify the basis cellular processes in cells derived from soft tissues sarcoma (STS, Rhabdomyosarcoma) with emphasis on its protective effects on normal muscle cells surrounding the tumor. **The hypothesis which will be verified in this project states that pulsed electrical field stimulation (PEF) in the presence of calcium ions will induce cell death by apoptosis or necrosis in rhabdomyosarcoma cells (RD) and stimulate normal muscle cells (C2C12) to increased physiological activity and proliferation.**

The research reveals the high potential for science development, because calcium signaling, which plays crucial role in numerous physiological processes, has not been sufficiently deepened yet. The pro-regenerative influence of an electric field on reconstruction of damaged muscle and nervous tissues is a well-known fact. We also know that electroporation (EP) is used for selective transport of small molecules into the cells e.g. in oncology as electrochemotherapy (ECT). On the other hand both opposite processes like **cell proliferation and cell death can be induced by modulation of intracellular calcium level**. The development of a **combined therapy based on calcium ions and EP seems to be extremely interesting and innovative approach**. The principles of mechanisms, which are activated by high calcium level and electric field have not been understood yet in the context of normal and pathological muscle cells.

The evaluation of the cytotoxicity after applied therapy with various parameters, two viability assays will be performed: MTT and SRB. The confocal microscopy and appropriate fluorescent dyes will be used to evaluate the impact of EP with calcium ions on cellular cytoskeleton (actin and tubulin fibers) and cell membrane, which is mostly exposed to PEF. In addition, calcium ions marker will allow tracking of the differences in calcium ions level before and after applied therapeutic intervention. The morphology of cell organelles after treatment will be assessed by the transmission electron microscopy (TEM). Particular attention will be focused on the cell membrane and calcium ions "exchangers", that are incorporated in membranes, on which therapy can have the most significant impact. Thus there will be evaluated the expression level of proteins building calcium channels and pumps, responsible for calcium homeostasis. Also the ratio of isoforms of myosin heavy chain (MHC) responsible for the repair processes in the muscles will be estimated in order to demonstrate the influence of treatment on regenerative capacity of normal cells. The assumption of the project is to develop protocols, that will enable **stimulation of normal cells with simultaneous destruction of cancer**. Thus there is planned an evaluation of cell death via immunocytochemical methods, western blot and fluorescent microscopy. The optimized protocols in vitro will enable the implementation of the second part of the project in murine model of subcutaneously injected tumors. The second part is planned in **collaboration with international partner which is a leader in electrochemotherapy in Denmark** (Harlev Hospital).

The characterization of the effects of external pulsed electric field in the presence of calcium ions will **expand the present knowledge** in the field of cellular interactions with calcium ions and electroporation mechanisms that are not fully understood. Calcium signaling and its duality manifests by inducing the physiological activity and death is extremely interesting in terms of **safe cancer therapy**. There are few reports on the action of calcium and EP in fibrosarcoma cells. The results will help to understand and explain the differences in performance of normal and cancerous cells and will create the basis for further research and development of new protocols for *in vivo* studies. The obtained in the framework of the project results will contribute to the development of protocols of **minimally invasive methods for difficult to treat pediatric rhabdomyosarcoma**.