Abstract

Cancer is a leading cause of death in the western world and is increasing in frequency world-wide. Although diagnosis, treatment and therapeutic approaches to cancer have improved, many types of cancer are still lethal due to the lack of radical treatment. One of the fatal neoplastic disease types with poor prognosis is represented by malignant mesothelioma (MM). MM is characterised by very high mortality rate and limited therapeutic options. The etiology of the disease is mainly associated with exposure to asbestos fibres. The incidence of MM is increasing in many countries. The search for novel molecular targets, anti-cancer strategies and drugs, which would considerably improve the treatment is of great importance. Certain new drugs, especially those with specific molecular targets, show high selectivity in their action to cancer cells, and have considerably increased the cure rate in some types of cancer. Mitochondria have recently emerged as a very promising target for anti-cancer agents. A group of compounds with anti-cancer activity that induce apoptosis by way of mitochondrial destabilisation, termed 'mitocans', have been a recent focus of research. Several mitocans have been shown to selectively induce apoptosis in cancer cells and suppress the growth of many types of carcinomas in pre-clinical models.

The aim of this PhD thesis was to study the pro-apoptotic effect of mitocans from the group of vitamin E analogues epitomised by the redox-silent α-tocopheryl succinate (α-TOS) and its modified form, mitochondrially targeted analogue of vitamin E succinate (MitoVES) on MM cells. The experiments were performed on MM cells due to the very aggressive nature of this treatment-resistent cancer. Both vitamin E (VE) analogues were known from previous studies to selectively induce apoptosis in cancer cells and suppress the growth of many types of carcinomas in pre-clinical models. At the molecular level they act as Bcl-2 homology domain 3 (BH3) mimetics and sensitise cancer cells to other drugs. More importantly, they induce apoptosis by affecting the mitochondrial complex II (CII) of the electron redox chain. Thereby they interfere with ubiquinone, the natural acceptor for electrons generated by the succinate dehydrogenase activity of CII during conversion of succinate to fumarate. The electron flow is disrupted and reactive oxygen species (ROS) are formed. These ROS diffuse into cytoplasm in the form of hydrogen peroxide and are converted into more reactive radical species that catalyse activation of the pro-apoptotic proteins, viz. oligomerisation of Bax or Bak, resulting in the formation of a megachannel in the mitochondrial outer membrane. This leads to the translocation of cytochrome c into cytosol via the channel, and the downstream mitochondrial pathway of apoptosis is initiated. In contrast to the untargeted prototypic VE analogue α-TOS, MitoVES has been modified with a triphenyphosphonium (TPP^+) group, which is important for the directed docking and accumulation of the compound in the mitochondrial inner membrane of a cancer cell, providing a much stronger apoptogenic efficacy.

In this work, it has been demonstrated that MitoVES is significantly more efficient in killing MM cells than α-TOS with IC_{50} lower by up to two orders of magnitude. Further, the mitochondrial accumulation of MitoVES in MM cells has been proved by confocal microscopy using its fluorescently tagged analogue (MitoVES-F). Shortening the aliphatic chain of MitoVES spanning the chromanol and TTP^+ groups has reduced the efficacy of the agent, suggesting that its possible target might be the mitochondrial CII. The function of CII in the activity of MitoVES has been cofirmed, as MM cells with suppressed

succinate quinone reductase were resistant to the agent. Further, MitoVES has induced apoptosis in MM cells via mitochondrial destabilisation, resulting in the decrease of mitochondrial membrane potential and generation of ROS. A mouse model has been used to show the superior anti-MM activity of MitoVES to α-TOS. All data obtained within this work have proven that mitochondrial targeting of vitamin E succinate endows the agent with considerably higher efficacy to kill MM cells, bringing it to the vicinity of its molecular target.

Another part of this PhD project involves a proteomic and genomic study of the influence of α-TOS on MM cells. Using 2-dimensional gel electrophoresis, a variety of up- or down-regulated proteins have been identified after the treatment of MM cells with the VE analogue. Some of these proteins have been confirmed by other techniques, including RT-PCR, real-time quantitative RT-PCR and western blot. We have focused on the cytoskeletal protein septin 11, which has been found to be down-regulated after the treatment with α-TOS by all used techniques. Unfortunatelly, the subsequent gene silencing experiments have failed. Due to the oncoming clinical trials (planned by our laboratory), there was a need to focus more on the project described above, i.e. the mechanims of killing of MM cells by MitoVES. Notwithstanding this focus, it is worth to complete the second part of this project, as the obtained results seem to be very interesting since septin 11 and the several other identified proteins have been described by previous studies to play important roles in a variety of functions of (cancer) cells, including regulation of apoptosis, cell proliferation, carcinogenesis and metastasis. It is planned that this part of the project will be completed in a follow up study in our laboratory.

The context of this work is within the potential cure of MM, a thus far fatal disease. Scientifically, it is believed that the acquired outcomes from this work will contribute to the emerging notion that mitocans hold a substantial promise to be developed into efficient anti-cancer drugs that will be utilised to help curb the increasing incidence of neoplastic diseases, as epitomised here by the extremely hard-to-treat mesothelioma.