Abstract (EN)

Analogously to normal stem cells within the tissues, cancer stem cells (CSCs) have been proposed to be responsible for maintenance and growth of tumours. CSCs represent a small fraction of cells within the tumour, which is characterised by self-renewal capacity and ability to give rise to a tumour when grafted into immunocompromised mice. Cells with increased stemness properties are believed to be responsible for tumour resistance, metastases formation and relapse after tumour treatment.

The first part of this work concentrates on resistance of the tumours, which is often associated with increased expression of ATP-binding cassete (ABC) transporters pumping chemotherapeutics out of the cells. For the purposes of this study, we utilized an *in vitro* model of CSCs, based on cultivation of cells as 3D "spheres". Expression profiling demonstrates that our model of CSCs derived from breast and prostate cancer cell lines express higher mRNA level of ABC transporters, particularly *ABCA1*, *ABCA3*, *ABCA5*, *ABCA12*, *ABCA13*, *ABCB7*, *ABCB9*, *ABCB10*, *ABCC1*, *ABCC2*, *ABCC3*, *ABCC5, ABCC8*, *ABCC10*, *ABCC11* and *ABCG2* among the cell lines tested. The protein level of ABC transporters tested in breast CSCs showed higher expression of ABCB8, ABCC1, ABCC2, ABCC10 and ABCG2 but downregulation of ABCB10 and ABCF2 proteins. Consistently, T47D and MCF7 spheres show resistance to daunorubicin and doxorubicin and interestingly, higher sensitivity to ABCC1 and ABCG2 inhibitors. These results suggest that ABC transporters may play an important role in maintenance of CSC phenotype unrelated to drug efflux.

The transition from oestrogen dependent to oestrogen independent tumour growth in breast cancer is associated with loss of oestrogen receptor α (ERα) and is connected with worse prognosis. This process might be regulated by microRNAs, 22 nucleotides long, single stranded, non-coding RNAs that negatively regulate gene expression by binding to mRNA, resulting in translation inhibition and mRNA degradation. We found that oncogenic microRNA-301a-3p (miR-301a-3p) is highly elevated in our *in vitro* model of breast CSCs, which show a decrease in ER signalling. We demonstrated that miR-301a-3p negatively regulates ER signalling by direct repression of ERα mRNA translation. High miR-301a-3p expression decreases the sensitivity of oestrogen dependent MCF7 cells to 17-β oestradiol and similarly inhibits growth of the tumour derived from this cell line in nude mice. Yet, the resulting tumours show significantly increased expression of genes related to CSCs and epithelial to mesenchymal transition suggesting for enrichment of CSCs population. Moreover,

miR-301a-3p expression negatively correlates with *ESR1* level in biopsies from breast cancer patients. Thus, miR-301a-3p may serve as a prognostic marker of poor patient prognosis, oestrogen independency and resistance to anti-oestrogenic drugs.

The last part of this work is focused on metabolism of iron in CSCs. Iron is indispensable micronutrient required as a cofactor for normal function of a plethora of proteins involved in cellular respiration, Krebs cycle, redox reactions as well as enzymes necessary for DNA replication and repair. Not surprisingly, deregulation of iron metabolism leads to many pathological situations including cancer. We show that MCF7 spheres exhibit higher labile iron pool, higher iron uptake with predominant mitochondrial iron accumulation and are more susceptible to iron chelation. MCF7 spheres also show activation of IRP/IRE system, explaining higher iron uptake and decrease in iron storage. Activity of iron sulphur cluster (ISC) containing enzymes in MCF7 spheres is lower suggesting for disruption of ISC machinery. Further, MCF7 spheres show higher oxidative environment reflected by higher level of reactive oxygen species and lower level of reduced glutathione. Gene expression profiling of CSCs derived from breast and prostate cell lines identified specific gene signature related to iron metabolism consisting of genes related to iron uptake (*CYBRD1*, *TFRC*), iron sensing and iron regulation (*ACO1*, *IREB1*), mitochondrial haem and ISC synthesis (*ABCB10*, *GLRX5*), hypoxia response (*EPAS1*, *QSOX1*), iron export and iron export regulation (*HEPH*, *HFE*), suggesting for profound changes in iron metabolism. Moreover, principal component analysis based on this signature is able to distinguish CSC from non-CSC population *in vitro*. Our findings show critical changes in iron metabolism related to CSC phenotype.

Altogether, our results point to a critical role of CSCs in tumour biology, highlighting differences between normal cancer cells and CSCs that could be potentially used for cancer diagnostics and therapy.