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Role of calretinin during mesothelioma development: influence on different signaling pathways, identification of new binding partners and evaluation of calretinin as a potential therapeutic target

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Malignant mesothelioma (MM) is an aggressive asbestos-related neoplasm that arises from mesothelial cells covering the surfaces of the pleura, peritoneum and pericardium. Pemetrexed and cisplatin combination chemotherapy remains as the only established treatment; however, benefits of this combination therapy still continue to be modest and the median survival time after MM diagnosis is less than 12 months. The prognosis is extremely poor due to a lack of understanding of MM biology and molecular pathogenesis. The Ca^{2+}-binding protein calretinin (CR) is currently used as a positive marker for human MM. Yet, the putative role(s) of CR during MM formation *in vivo*, binding partners or CR's influence on specific signaling pathways remain unknown.

The effect of CR overexpression in different MM cell lines was evaluated *in vitro*. We found that CR increased the migration and invasion of MM cells *via* the activation of the FAK signaling pathway. In addition, the protein FAK was identified as a new binding partner of CR. The discovery of new binding partners of CR confirms previous findings that CR not only acts as a Ca^{2+} buffer, but also as a Ca^{2+} sensor protein. CR was also implicated in controlling epithelial-to-mesenchymal transition (EMT) and in increasing the resistance of MM cells towards the FAK inhibitor VS-6063 and towards cisplatin.

As *in vitro* downregulation of CR was previously shown to decrease the proliferation and viability of MM cells, we generated a tightly controlled IPTG-inducible system to manipulate CR expression levels *in vitro* in a time-dependent manner to evaluate the early molecular events occurring after CR downregulation. Cells with decreased CR expression levels showed a significant decrease in viability and proliferation, an attenuation of the FAK signaling pathway and a reduced invasive phenotype. In addition, CR downregulation augmented the sensitivity of the cells towards the FAK inhibitor VS-6063. However, the few surviving cells showed an augmented resistance to cisplatin. It is well known that many tumor cells become resistant to cisplatin, primarily by inactivating apoptotic factors and/or enhancing cell survival pathways including the PI3K/AKT, FAK and Wnt/β-catenin signaling pathways. A specific PCR array revealed an increase in genes related to the Wnt signaling pathway. Using the Wnt inhibitor 3289-8625, we demonstrated that the initial observed chemoresistance was abrogated, confirming a direct role of the Wnt signaling pathway in mediating the cisplatin resistance of the MM cells.

Finally, in an orthotopic xenograft mouse model based on peritoneal MM cell injection, CR downregulation *via* a lentiviral sh*RNA* against CR (*CALB2*) resulted in a significantly reduced tumor formation *in vivo*. Nonetheless the detailed mechanisms by which CR downregulation decreases tumor progression still needs to be further elucidated. Having demonstrated the important functions that CR exerts during MM development, targeting CR in combination with other components of key signaling pathways (such as FAK or Wnt) emerge as a valuable and promising therapeutic option for MM treatment.

Jury: Prof. Dr. Beat Schwaller (PhD thesis Supervisor)
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