9th WORLD CONGRESS ON
IMMUNOLOGY AND CANCER

December 09-10, 2019 | Barcelona, Spain

Cell-cell and cell-substratum contacts: Impact on MAPK signaling molecules involved in the regulation of cancer and stem cell functioning

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Background: Anoikis ("homeless") is an apoptotic form of programmed cell death induced by detachment from the extracellular matrix. Resistance to anoikis is emerging as a hallmark of cancer. During anticancer therapy, cell-cell and cell-substratum contacts play important roles in the cell fate determination. Increased resistance to anoikis enables malignant cells to survive during migration into secondary tissue and metastasize. Normal cells, however, do not possess such resistance. This is why molecular mechanisms of anoikis regulation may serve as a target both in reducing metastatic cancer growth and in increasing stem cell therapy efficacy. Superfamily of Mitogen-Activated Protein Kinases (MAPKs) regulates cell functions such as proliferation, differentiation and programmed cell death. The regulatory events initiated by extracellular contacts also involve MAP kinase cascades. In the present study, the impact of extracellular contacts on activation of signaling molecules AKT, MAPKs (ERK, JNK, p38) as well as transcription factor cJun in different mammalian cell *in vitro* model systems – lung adenocarcinoma A549, primary lung cancer cell and muscle-derived stem cell lines – was investigated.

Methods: Human non-small cell lung cancer adenocarcinoma A549 cells were obtained from Cell Lines Service (Germany). Human primary lung cancer cell lines were established from surgical material (regional bioethical approval no. 158200-18/5-1024-537). Myo stem cell line was derived from adult rabbit thigh muscle anterior tibia in Institute of Biochemistry (Lithuania). Cells were cultivated at 37ºC and 5% CO2 in IMDM medium supplemented with 10% FBS and antibiotics.Inhibition of cell adhesion. Trypsinized cells were suspended in CO2-independent medium and incubated in a shaker for 24 hours. Then cells in suspension were fractionated in order to obtain non-aggregated and aggregated cell fractions and lysed for protein analysis by Western blot method. Mode of cell death was determined by fluorescence microscopy using a mixture of acridine orange/ethidium bromide fluorescent dyes to identify the apoptotic (anoikis) cells. The role of specific signaling pathways during anoikis was evaluated using specific inhibitors: LY294002 (PI3K/Akt pathway), AZD6244 (ERK pathway), SP600125 (JNK pathway), PF573228 (FAK inhibitor) and MG132 – proteasomal inhibitor.

Findings: The results indicate that cancer cells are more resistant to anoikis when compared to stem cells. Cells in aggregates survived better than single cells in suspension. Survival of both kinds of cells was dependent on protein kinases AKT and ERK. Anoikis diminished AKT phosphorylation in both kinds of cells, whereas phosphorylation of p38 and JNK increased. However, loss of cell-substrate contacts differently affected ERK activation in tested cancer and normal stem cells. Cell-substrate-independent ERK activation was observed in oncogenic RAS harboring A549 cells.

In addition, we have demonstrated that expression of transcription factor c-Jun was dependent on cell culture density in a way that cell-cell contacts promoted proteasomal degradation of c-Jun. Furthermore, phosphorylation of prosurvival kinases AKT and ERK increased during myogenic differentiation of Myo cells making them more resistant to anoikis.

Conclusion & Significance: The results suggest that targeting of prosurvival kinases AKT and ERK during anoikis should be different in different cancer and stem cells. Manipulation of MAPK activity could be engaged in the improving efficacy of cancer therapy.

Acknowledgement: This work has received funding from the European Regional Development Fund (Project No. 01.2.2-LMT-K-718-01-0072) under grant agreement with the Research Council of Lithuania (LMTLT).

Biography

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