Volume 57(1):83-94, 2013
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Examination of melanoma tumor-host relationship

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Metastatic melanoma (MM) is an especially aggressive skin cancer. The mechanism of its rapid metastasis formation, high genetic variability and effective immune escape mechanisms are not explained yet. The NF-κB signal pathway plays a complex and major role in malignant diseases. Up-regulated NF-κB activity is frequently detected in various tumors, and it is implicated in many facets of the malignant behavior, including tumor immune escape, invasion, angiogenesis and metastasis, and chemotherapy resistance. Chemotherapy might further increase the elevated NF-κB activity of tumor cells, thus protecting them from chemotherapy-induced cell death. Among the potential mediators, tumor-derived exosomes might also contribute to tumor escape.

Our first hypothesis was that NF-κB inhibitor vanillin analogs might have an anti-tumor effect, and they may also synergize with anti-cancer drugs. Blocking of the NF-κB signal pathway by vanillin analogs might have a complex anti-tumor effect. In the first step of our project, we have tested the *in vitro* cytotoxicity and NF-κB inhibitory effect of a panel of vanillin analogs on the B16 mouse melanoma cell line. Based on the results, we have selected the most promising analog, ortho-vanillin (QL7), for the *in vivo* experiments. Testing the second hypothesis, we explored the interaction of melanoma cell derived exosomes (mcde) and their microenvironment. We investigated how mcd-exosomes influence CD4+ T cell proliferation induced by bone marrow derived dendritic cells. We quantified the NF-κB activation in mature macrophages stimulated with mcd-exosomes, than we analyzed their cytokine/chemokine profile.

In *in vitro* cytotoxicity assay we found that the vanilloid analogue QL7 reduces cell proliferation after 48h incubation. Treating the cells with QL7 plus doxorubicin induced cell proliferation, while the doxorubicin-induced by 6 h NF-κB activation was suppressed by QL7. In animal experiments we found that the QL7 administered together with cyclophosphamide reduces the primary tumor size. We observed that mcd-exosomes help the maturation of dendritic cells, and enhance T cell proliferation induced by the treated dendritic cells. The exosomes also activated macrophages, as measured by NF-κB activation. The cytokine and chemokine profile of macrophages treated with mcde showed marked differences from those induced by either LPS or IL-4.

Our results suggest that 1) NF-κB inhibitor vanillins have an anti-tumor effect, and 2) exosomes may play a role in the tumor progression and metastasis via supporting tumor immune escape mechanisms.

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Characterization and identification of the molecular interaction partners of an actin regulating protein

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The formin proteins are an important and evolutionarily well conserved class of actin binding proteins with essential biological functions, including cell division, cell migration and organelle transport. In these processes the best understood molecular role of formins is to promote the nucleation and elongation of unbranched actin filaments, although some formins have also been implicated in the regulation of microtubules. We have previously shown that the single *Drosophila* DAAM ortholog, dDAAM, is involved in multiple aspects of tracheal development and axonal growth regulation, however the molecular mechanisms underlying these morphogenetic functions remain to be uncovered. To gain a better understanding of the molecular functions of dDAAM, we aim to identify the protein interaction partners of dDAAM with biochemical and genetic methods. The biochemical interaction partners are aimed to be identified by affinity chromatography. To this end, we created a dDAAM-Flag fusion protein by tagging the *dDAAM* gene *in situ* by site specific mutagenesis. The purification of the dDAAM containing protein complex is carried out from the adult head that is easy to isolate and, based on our former observations, dDAAM is highly enriched in the brain.

Besides the interaction partners, we are also interested in the functional characterization of dDAAM. During the investigation of the $dDAAM^{Flag}$ mutant strain we revealed the existence of a novel dDAAM isoform (dDAAM-PD) that is absent from the brain but enriched in muscles. Because *dDAAM* plays an important role in sarcomere formation, it is of main interest to understand the functional properties of dDAAM-PD that appears to be the major muscle isoform. To this end, we created *dDAAM-PD* specific overexpression and RNAi tools that will hopefully allow us to determine how this isoform contributes to the development of muscles and other tissues.

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