Analysis of Glypican Proteoglycans and *Wnt* Signaling Pathway: Role in Prostate Cancer.

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Introduction: Glypicans are cell surface proteoglycans linked to the membrane through glycosyl-phosphatidylinositol anchor. **Objectives**: Wnt-mediated signaling was correlated with the glypican expression in the pathogenesis of human prostate cancer. **Material and Methods**: Human prostate adenocarcinoma cell lines LNCaP, PC3 and DU145 were used compared to the normal RWPE-1 cells. **Results and Discussion**: Glypican expression pattern was analyzed by RT-PCR and showed that glypican-1 was expressed at high levels by the cells, with exception of LNCaP cells which express mainly glypican-5. Glypican-1 was investigated by immunocytochemistry, whose subcellular localization was achieved at the cell surface in RWPE-1 and PC3 cells. Interestingly, in DU145 glypican-1 was found in cytoplasmic compartment. Analysis by flow cytometry confirmed glypican, Wnt3a ligant and activated β-catenin in the cell lines. Density plot of glypican-1 and Wnt3a in RWPE-1, PC3 and DU145 cells shows a double labeling population of 9,4%, 5,1% and 12,5%, respectively. Interestingly, the density plot of Wnt3a and activated β-catenin in cancer cells showed an increase in double labeling of these two molecules, compared to normal RWPE-1 cells, suggesting a correlation between the ligant and the final element of Wnt signaling pathway, β-catenin. In order to investigate the involvement of glypican in this pathway, co-immunoprecipitation was done and Wnt3a in the precipitated material was analyzed by western blot. Increased levels of these proteins were detected in cancer cells, suggesting the link of glypican with this pathway. Translocation of membranous β-catenin to the nucleus has been associated with progression to malignant prostate carcinogenesis. So, nuclear and cytoplasm β-catenin were investigated and a markedly increase in nuclear β-catenin was observed in cancer cell lines, compared to RWPE-1 cells. **Conclusions**: Glypican expression in prostate cancer cell lines correlates with activated nuclear β-catenin via Wnt3a ligant and has the potential to interfere with the canonical Wnt signaling pathway, events which control neoplastic growth and disease progression.

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