Targeting urokinase plasminogen activator receptor (uPAR)/integrin complex formation, a signaling orchestrator in cancer

Der Urokinase Plasminogen Aktivator (uPAR)/Integrin Komplex als Koordinator der Signaltransduktion in Krebszellen

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Ausgangssituation

The urokinase plasminogen activator receptor (uPAR) / plasminogen system is highly expressed in a variety of tumor types, thereby it affects tumor cell behavior. It's functional role is not only limited to it's proteolytic activity, but also mediated via intracellular secondary signaling events. As uPAR is a GPI-anchored protein, lacking a transmembranous domain, lateral interaction partners such as integrin adhesion receptors or members of the LDLR-family are required. Recently, we demonstrated that uPAR becomes redistributed to the leading edge of migrating cells upon VEGF stimulation via a coordinated internalization and recycling mechanism, which involves uPA:PAI-1:uPAR complex formation and LDLR-family member mediated complex internalization. There is increasing evidence that uPAR directly interacts with LDLR family members via a so far unidentified binding motif, thereby linking integrin redistribution to the LDLR internalization receptor.

Ziele / Methoden

As part of Aim 1, we have now characterized an LDLR binding motif on uPAR domain 3. By in silico protein structure prediction (Figure 1), generation of single amino acid mutations as well as affinity chromatography experiments, we identified a responsible binding motif for direct LDLR interaction by introduction of a flexible linker sequence (GGLG) substituting the amino acid residues forming loop 1 (K197G, N199G, H202L) in uPAR. By expression of either mutated uPAR or wild type uPAR into uPAR -/- HEK 293 cells, we studied the functional and biological consequences of suspended uPAR / LDLR family interaction. Additionally, we used a competitive interfering peptide (P1) mimicking the binding domain of uPAR as well as chaperone Receptor Associated Protein (RAP)-mediated inhibition of ligand binding to LDLR family members: Suspended uPAR / LRP interaction thereby led to reduced migratory response towards a variety of different pro-migratory stimuli (Figure 2). From these data we conclude that uPAR / LDLR-interaction is required for efficient pro-migratory cell behavior. Furthermore, interaction with uPAR / LDLR / integrin complex formation led to a decrease in integrin-induced signal transduction such as pY576 FAK phosphorylation (Figure 3) most likely due to reduced integrin redistribution. That uPAR / LDLR interaction is essential for efficient cell migration was further supported by results obtained by randomized migration assays using ither wild

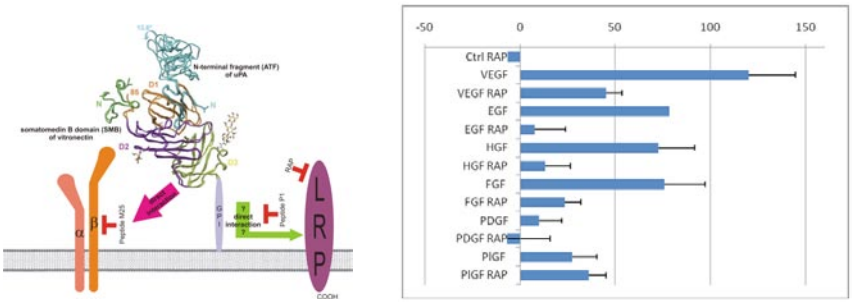

Abb. 2: Cell Migration upon a variety of different stimuli depends on direct LDLR-like protein interaction as addition of RAP could reduce the migratory response towards VEGF, EGF, HGF, as well as FGF-2, while PDGF or PlGF had only minor effects on endothelial cell migration.

type of uPAR-/- endothelial cells. Thereby, VEGF was less effective whenever uPAR was absent and interference with LDLR binding could block VEGF-induced endothelial cell migration whenever uPAR was present. From these data we conclude that efficient endothelial cell migration requires uPAR / LDLR interaction.
The biological relevance of targeting VEGF-induced cell migration was further analyzed by experiments using a specific monoclonal antibody directed against VEGF, bevacizumab. Addition of this antibody to VEGF-induced tumor cell transmigration led to blockage of cancer cell transmigration through an endothelial monolayer.
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Ergebnisse

We have succesfully completed Aim1 and Aim 2 of the study supported by Initiative Krebsforschung and are currently preparing completion of Aim 3. Results from this study have already been published or are in preparation for publication in international peer-review journals.
Furthermore, data generated by this project have been selected for presentation at international meetings, such as Gordon Research Conference in Ventura, California (02/2010), International Vascular Biology Meeting, Los Angeles (06/2010), as well as International Society of Fibrinolysis and Proteases, Amsterdam (08/2010).

Ausblick

Our group has applied for a Stand Alone project at FWF for continuing the research on this field.

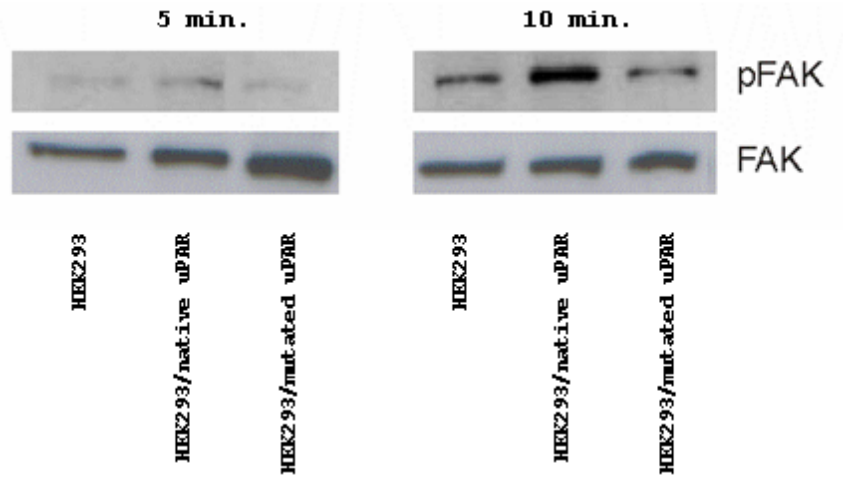

Abb. 3: Western blots: HEK 293 cells expressing either native uPAR or mutated uPAR or mock as control, showed that uPA-induced FAK phosphorylation, whih is the most upstream signalling event of integrin-induced signal tranduction requires direct uPAR / LDLR-interaction.

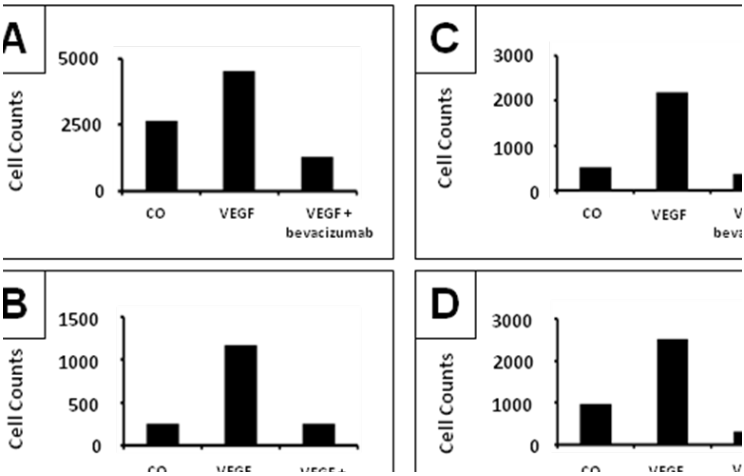

Abb. 4: uPAR / LDLR interaction seems to be required for efficient cell migration as interfering with complex formation by RAP blocked the migratory response towards VEGF. Furthermore, in uPAR deficient ECs VEGF was less effective, but still significant, but was not affected by interference with LDLR-interation.

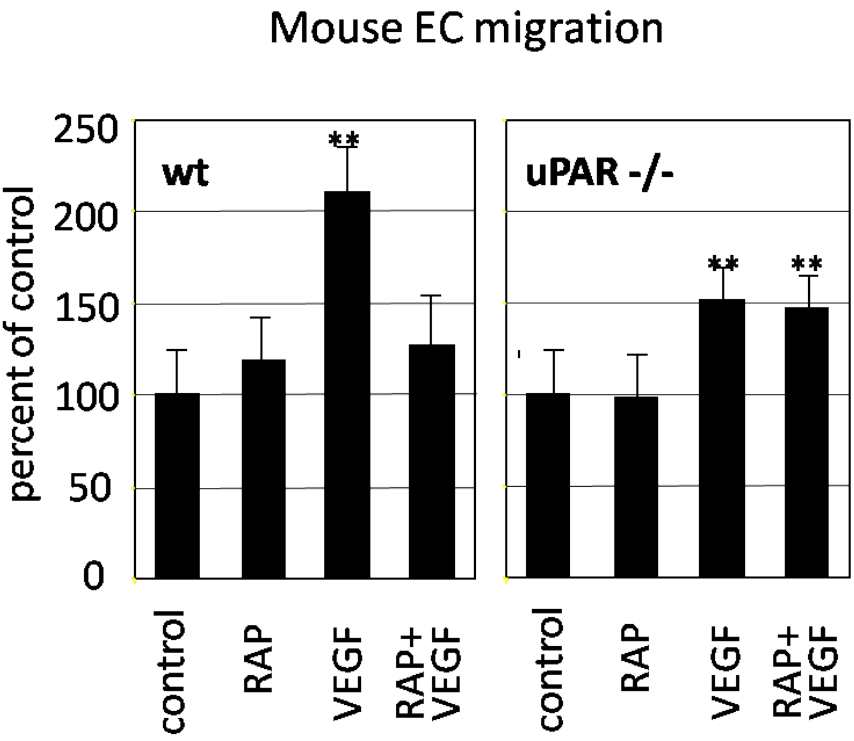

Abb. 5: A-D: Effects of recombinant VEGF165 on trans-endothelial migration of primary tumor cells. Biotinylated primary tumor cells from two patients with lung carcinoma (A: #12 and B: #13 in Table 1), one patient with gastric carcinoma (C: #14), and one with breast carcinoma (D: #15) were placed into the upper chambers. Control medium (CO) or recombinant VEGF165 (50 ng/ml) with or without bevacizumab (400 ng/ml) were placed into the lower chambers as indicated. Tumor cells were allowed to transmigrate through endothelial monolayers at 37°C for 24 hours. Thereafter, migrated cells were recovered and counted by microscopy. Results show the number of migrated cells under each condition (each one experiment per donor).