to examine the effect of VEGF-D on the expression of genes associated with disease progression in VEGFR-3-expressing KKLS cells. To stimulate VEGF-D/VEGFR-3 autocrine signaling, a VEGF-D expression vector was transfected into KKLS cells, and stable transfectants were established. VEGF-D-transfected cells and control cells were then transplanted into the gastric wall of nude mice (i.e., orthotopically).

Results: Gastric carcinoma cell lines constitutively expressed VEGF-D mRNA. Two of the four cell lines expressed VEGFR-3 mRNA and protein. In vitro treatment of KKLS cells with exogenous VEGF-D stimulated cell proliferation and increased expression of mRNAs encoding Bcl2 and autocrine motility factor receptor. Proliferation of VEGF-D-transfected cells transplanted into the gastric wall of nude mice was greatly increased compared to that of control cells. VEGF-D transfection into KKLS cells resulted in inhibition of apoptosis and stimulation of angiogenesis and cell proliferation. However, lymphangiogenesis was not increased in response to VEGF-D transfection.

Conclusions: Human gastric carcinoma cell lines express VEGF-D and VEGFR-3. VEGF-D may be involved in the progression of human gastric carcinoma by acting via autocrine and paracrine mechanisms.

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Monitoring of tumor progression using bioluminescence imaging in a nude mice orthotopic model of human small cell lung cancer

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Background: Lung cancer is the main cause of cancer deaths throughout the world and a clinically relevant animal model of human small cell lung carcinoma (SCLC) should be useful to study the molecular aspects of the tumor progression and test the efficiency of new therapeutic agents. In this study, we generated a reproductible and reliable nude mice orthotopic model of human SCLC based on NCI-H209 tumor cells genetically modified to express firefly luciferase. Materials and Methods: NCI-H209 cells were transfected with pCMV-luc plasmid and clones highly expressing luciferase were isolated and amplified. Cells were analyzed for long-term bioluminescent stability and a clone was subcutaneously passaged twice in vivo to enhance tumorigenicity. Cells resuspended in Matrigel® and/or EDTA RPMI medium containing a Tc99M colloid were implanted intrabronchially using a catheter inserted into the trachea and positioned into the right main bronchus using interventional imaging. Punctual deposition of cells was then assessed by scintigraphy. Results: Only tumor nodules were observed into lung and trachea when cells were implanted with EDTA. Lung tumor invading parenchyma were present in 40% of the mice with Matrigel® and improved to 75% with EDTA and Matrigel®. The growth of the primary tumor was sensitively and non-invasively followed and quantified by bioluminescence imaging using a CCD-camera. This tool allows a real-time monitoring of tumor progression on the same animals over a 2-12 week period. Combination of 3D bioluminescence imaging and computed tomography scanning was used to further document tumor location and measurement. Subsequently, the histological analysis of tissue sections confirmed the presence of a lung tumor. Conclusion: Our nude mice orthotopic model resembles various stages human small cell lung carcinoma, and then could be useful for evaluating new therapeutic strategies.

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Voltage-gated sodium channels activity promotes cysteine cathepsins-dependent invasiveness of human cancer cell lines

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Background: Various molecular isoforms of voltage-gated sodium channels (Na_v) are functionally expressed in several cancer types of non-excitable, epithelial tissues (breast, prostate, lung). In the aggressive breast cancer cell line, MDA-MB-231, we found that the $Na_v1.5$ isoform is involved in cell invasiveness. Our goal is to understand the link between Na_v activity and extracellular matrix proteolysis.

Materials and methods: To study the activity of the protein, we used the patch clamp technique in the whole cell configuration. RT-PCR and western-blot were used to identify the isoforms of the different proteins studied. The regulation of genes transcription was studied by quantitative PCR. Enzymes activities were determined using fluorogenic peptidyl substrates. Intracellular pH was monitored using the ratiometric fluorescent dye BCECF and cell surface pH using fluorescein-conjugated DHPE (N-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine).

Results: The activity of Na_v promoted the invasive properties of cancer cells. The inhibition of Na_v by the specific blocker tetrodotoxin (TTX) impaired the invasivity of MDA-MB-231 cells. These cells express different functional cysteine cathepsins. Matrigel™ invasion was decreased by 65% in presence of a broad spectrum, membrane-impermeant inhibitor of cathepsins, and was specifically decreased by inhibitors of cathepsins B and S (CA-074 and Z-FL-COCHO). The association of these inhibitors with TTX demonstrated no further effect, indicating the regulation of extracellular cathepsins activity by Na_v. Blockade of $Na_v1.5$ activity by TTX for 24h had no effect on the transcription of genes encoding for cathepsins, cystatins (cathepsins endogenous inhibitors) or $Na_v1.5$. Likewise, no difference in the amount of cysteine proteases released in the extracellular medium was observed by western blot or by enzymatic titration assay, indicating that Na_v does not influence secretion and membrane-associated cathepsins activity. Conversely, we found that Na_v activity leads to an intracellular alcalinization and thus participates in the acidification of the pericellular space. Such an acidification is favourable to the activity of cysteine cathepsins.

Conclusion: This work suggests that Na_v activity facilitates invasiveness of cancer cells by promoting pH-dependent activation of cysteine cathepsins.

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Expression of the carboxy-terminal tail of connexine 43 could induce similar effects to full-length connexin 43 on tumor proliferation

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Background: Accumulating data seem to associate connexin 43 (Cx43), a structural protein of Gap Junction Intercellular Communication (GJIC) and tumorigenicity. The aim of our study was to establish the relative importance of GJIC versus the intracellular signaling pathways mediated by the carboxyl tail (C-tail).

Materials and methods: LN18 human glioma cell line expressing low levels of endogenous Cx43 was transduced by retroviral infection with different forms of Cx43: (1) the full-length Cx43, (2) a truncated TrCx43 lacking the C-tail, and (3) the carboxyl tail only, 243-Cx43. Proliferation rate was determined by cell counting, migration behavior was studied by wound healing assay and transwell assay; and oncogenicity was examined by anchorage-independence soft agar assay.

Results: As expected, Cx43 was localized to the cell membrane in LN18-Cx43 cells. LN18 TrCx43 showed a membranous and cytosolic Cx43 staining and LN18 243-Cx43 exhibited a diffuse signal. Increased number of coupled cells (28±4 coupled cells versus 10±2 for LN18 mock) was detected only in LN18-Cx43 but not LN18-TrCx43, indicating that the C-terminal tail of Cx43 is needed for optimal GJIC. There is no significant difference in growth rate between these three lines on monolayer cultures. Interestingly, expression of all three Cx43 constructs reduced the oncogenicity of LN18 cells – they had a lower number of colonies and smaller colonies than LN18-mock in soft agar. Un-expectably, all three Cx43 constructs were equally well in increasing migration rate in wound healing assays (the transfected LN18 lines expressing different Cx43 constructs moved over 200 μm in 24 hours versus 150 μm for the LN18 mock).

Conclusions: Taken together, these results indicate that 1) GJIC is not required for Cx43-dependent cell motility, 2) the similarities in cellular effects observed with TrCx43 and 243-Cx43 suggest that Cx43 affect cell motility and growth by affecting two independent pathways. There is already evidence to suggest the extracellular domain of Cx43 is important for adhesion (Elias et al, 2007). Although our results indicate that a role of C-tail in modulating GJIC, a more important function of the C-tail of Cx43 in modulating tumorigenicity appear to be acting as a scaffold, bringing many signaling molecules together in close proximity.

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Host cell recruitment by gliomas

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Malignant gliomas are the most common primary brain tumors and are considered among the deadliest of human cancers. Molecular, cellular and genetic analysis has advanced our understanding of these tumors, but little is known about the responses of the host brain and other organs to gliomas. Data suggest a two-way cell trafficking between tumor and host;