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;

glioma cells spread into the normal brain parenchyma, whereas numerous cell types are recruited to the tumor.

We studied orthotopic implants of human glioma cell lines (U251, U87, D566) in nu/nu mice and human primary glioma in nu/nu rats. Glioma implants attracted large numbers of host nestin+ cells. Double-labeling for human and mouse nestin revealed an interdigitated network of tumor and host cells in glioma mass and at invasive tumor edge. PCNA immunostaning revealed areas of proliferative activity within glioma, as well as in ipsilateral SVZ. GFAP+ cells formed a distal halo around the primary tumor site, whereas Ms nestin+ cells surrounded the glioma proximally and penetrated into the tumor mass. The glioma mass displayed high levels of angiogenesis. Invasive glioma micro-foci (< 100 micrometers) contained Ms nestin+ cells with arbor-rich morphology, but these small tumor foci lacked blood vessels. SMA+ cells, which are likely vascular mural cells (pericytes or smooth muscle cells) were present within primary glioma mass and surrounding tumor foci. Ms nestin+ cells were also present at sites of vessel sprouting and bifurcation, suggesting a role of these cells in vessel formation. Close physical contact was apparent between Ms nestin+ and SMA+ cells during glioma neovascularization, a phenomenon known to be associated with TGF-beta signaling and endothelial cell-directed differentiation of mesenchymal cells into mural cells. In intracranial implants of primary human glioma in nu/nu rats, we detected distinct tumor phenotypes at each passage. Highly invasive, non-angiogenic tumor was associated with low passage number (1st), and less invasive, highly angiogenic phenotype was associated with higher (5th) passage number. In the latter, we observed Rat nestin+, glomerulus-like blood vessels, which recapitulated the morphology of malformed vasculature observed in patients with high-grade glioma. SDF-1 and its receptor CXCR4 were highly expressed in and around glioma, which may be involved in both tumor invasion and attraction of host cells. SDF-1 was also expressed on tumor-associated blood vessels, where it may serve as 'trap' for circulating cells, including cells derived from the bone marrow.

Our data suggest that glioma and host brain are connected by an intricate network, which includes recruitment of host cells to the tumor. Defining the role of recruited cells in the biology of gliomas may aid the development of novel anti-glioma therapies.

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The role of translationally controlled tumour protein in tumourigenesis

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Background: Translationally controlled tumour protein (TCTP) is a highly conserved protein with numerous functions, including a role in cancer. It has been shown to interact with, and inactivate, the translation factor eEF1A (eukaryotic elongation factor 1A). However, it is unclear whether it binds to eEF1A1, the commonly expressed form of this protein, alone, or whether it also binds to eEF1A2, which has a more restricted expression pattern. The inappropriate expression of eEF1A2 in tissues in which it is not normally expressed is associated with cancer. If TCTP binds to eEF1A1 only, and not eEF1A2, then this would provide a possible explanation for the oncogenicity of eEF1A2.

Materials and Methods: Co-immunoprecipitation was used to pull down TCTP, and also any proteins with which it interacts. Antibodies specific for eEF1A1 and eEF1A2 were then used to determine to which translation factor TCTP binds.

Immunohistochemistry was also used on tissue microarrays, to determine whether TCTP is upregulated in cancer.

We have also used short interfering RNAs (siRNA) to knock down the expression of TCTP in different cell lines.

Results: 1. TCTP binds to both eEF1A1 and eEF1A2. 2. TCTP is upregulated in a high proportion of tumours. 3. We have successfully knocked down TCTP in different cell lines.

Conclusions: As TCTP binds to both eEF1A1 and eEF1A2, it is unlikely that the reason eEF1A2 is upregulated in cancer is due to its inactivation by TCTP. Additionally, we have confirmed a role for TCTP in cancer, as it is upregulated in tumour samples compared with normal tissues. Ongoing experiments will determine the effect on cellular proliferation and cell architecture when TCTP is knocked down.

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Positional cloning of t(3;6) in rat endometrial cancer

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Cytogenetic aberration and chromosomal rearrangement are common features of tumors. The consequence of balanced rearrangements can be deregulation of genes in the breakpoints or formation of a new gene, a fusion gene. Fusion genes may provide favorable properties to the tumors thereby paving the way to full malignancy. Expression of fusion genes is of decisive importance for diagnosis, classification, prediction of clinical outcome, and choice of therapy. Cytogenetic analysis and Spectral Karyotyping (SKY) analyses of 23 endometrial adenocarcionoma (EAC) tumors developed in females from BDII rat strain derived crosses illustrated that translocations involving rat chromosome (RNO) 6 were common among these tumors. Two tumors showed a similar translocation between RNO3 and RNO6, t(3;6) and a third tumor showed a complex form of translocation t(3;6) with a ladder like pattern in form of exchange of multiple chromosomal segments between these two chromosomes. In addition, yet three other tumors displayed translocations involving RNO6 fused with RNO10 or RNO16. Using Fluorescence in situ Hybridization (FISH) on metaphase spreads from these six tumors and DNA from BAC (bacterial artificial chromosome) clones as probes, positions of the chromosomal breaks in translocation events were determined. In dual-color FISH, we could successfully show that t(3;6) breakpoints in RNO3 and RNO6 were identical in two tumors, NUT97 and NUT98. In addition, FISH analysis revealed that RNO6 breakpoints in the other four tumors were not similar to that observed in NUT97 and NUT98, but derive from approximately the same region at the distal part of RNO6. This part of RNO6 is homologous to distal human chromosome 14q, which has been reported to be involved in balanced chromosomal aberrations in adenocarcionoma tumors in the ovary. These results may provide new insights into pathways involved in endometrial carcinogenesis.

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The involvement of wnt beta-catenin signal pathway in the invasion and the migration of oral squamous cell carcinoma cells

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Background and purpose: The Wnt signal pathway is involved in the carcinogenesis of various tumors including oral squamous cell carcinoma (SCC). In the presence of Wnt signals, Wnt receptors, which are frizzled homologs, activate the phosphoprotein Dishevelled and the ability of GSK-3beta to phosphorylate beta-catenin is then inhibited. Unphosphorylated beta-catenin is stable and accumulates in the cytosol and nucleus. In the nucleus, beta-catenin binds to T-cell factor (TCF)/lymphocyte enhancer-binding factor (LEF) to form a functional transcription factor which mediates the transactivation of target genes such as c-myc, cyclin D1, c-jun, fra-1, and u-PAR. In this results, invasiveness and migration increase in many kinds of tumor cells. We have reported the cytoplasmic and nucleous accumulation of beta-catenin in oral SCC. Then, we investigated the influence of the cytoplasmic and nuclear accumulation of beta-catenin on the oral SCC cell. Materials and methods: Oral SCC cell line were used in which beta-catenin expressed in the membrane but not in the cytoplasm and nuslei. Wild type beta-catenin cDNA containing the entire cording region and a mutated form of beta-catenin cDNA lacking exon3 including specific GSK-3beta phosphorylation sites were cloned into pUHD10-3 vector under regulation of a tetracycline-responsive promoter.These cDNAs was each cotransfected with pUHD15-1Neo, and stable cell lines were established. Results; Immunohistochemical staining using anti-beta-catenin antibody confirmed accumulation of beta-catenin in cytoplasm and nuclei of transfectants. In invasion assay and migration assay, invasion and migration activity of transfectants much more increased than those of parental cell line.Then, the transcriptional activity of Tcf DNA binding sequence in transfectants more increased than those of parental cell line. Then doxycycline reduced this activity. MMP-7 expression level of mRNA and activity of transfectants more increased than those of parental cell line. Further, Rearrangement of cytoskeleton protein and increase of activity of Rho family were observed in transfectants.

Conclusions; We suggested that in oral SCC cytoplasmic and nuclear accumulation of beta-catenin induced the increase of invasion and migration activity partially interacted with Tcf/lef transcriptional activity and partially through the rearrangement of cytoskeletal proteins and the activation of Rho family. Therefore, the malignancy of oral SCC increased interacting with wnt beta-catenin signaling pathway.

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Modulation of cellular response to stable RNA silencing of tissue factor pathway inhibitor-2 in lung cancer cells

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Introduction: Lung cancer is frequently diagnosed at an advanced stage and the malignant potential of this cancer depends on the ability of tumor cells to invade the surrounding tissue and form metastasis. This invasion