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