Brain Cancer

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Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, cancers can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the brain cancer.
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1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death.

The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

Literatures

Aronica, E., J. A. Gorter, et al. (2005). "Localization of breast cancer resistance protein (BCRP) in microvessel endothelium of human control and epileptic brain." Epilepsia **46**(6): 849-57.

Breast cancer resistance protein (BCRP) is a half adenosine triphosphate (ATP)-binding cassette (ABC) transporter expressed on cellular membranes and included in the group of multidrug resistant (MDR)-related proteins. Recently, upregulation of different MDR proteins has been shown in human epilepsy-associated conditions. This study investigated the expression and cellular distribution of BCRP in human control and epileptic brain, including a large number of both neoplastic and nonneoplastic specimens from patients with chronic pharmacoresistant epilepsy. Several epileptogenic pathologies, such as hippocampal sclerosis (HS), focal cortical dysplasia (FCD), dysembryoplastic neuroepithelial tumor, oligodendroglioma astrocytoma, and glioblastoma multiforme were studied by using Western blot and immunocytochemistry. With Western blot, we could demonstrate the presence of BCRP in both normal and epileptic human brain tissue. In contrast to P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP) 2, BCRP expression levels did not change in tissue from patients with HS, compared with control hippocampus. No BCRP immunoreactivity was observed in glial or neuronal cells, including reactive astrocytes and dysplastic neurons in FCD. BCRP expression was, however, increased in tumor brain tissue. Immunocytochemistry demonstrated that BCRP was exclusively located in blood vessels and was highly expressed at the luminal cell surface and in newly formed tumor capillaries. This localization closely resembles that of P-gp. The higher expression observed in astrocytomas by Western blot analysis was related to the higher vascular density within the tumor tissue. CONCLUSIONS: These results indicate a constitutive expression of BCRP in human endothelial cells, representing an important barrier against drug access to the brain. In particular, the strong BCRP expression in the microvasculature of epileptogenic brain tumors could critically influence the bioavailability of drugs within the tumor and contribute to pharmacoresistance.

Arrieta, O., D. Saavedra-Perez, et al. (2009). "Brain metastasis development and poor survival associated with carcinoembryonic antigen (CEA) level in advanced non-small cell lung cancer: a prospective analysis." BMC Cancer **9**: 119.

Central nervous system is a common site of metastasis in NSCLC and confers worse prognosis and quality of life. The aim of this prospective study was to evaluate the prognostic significance of clinical-pathological factors (CPF), serum CEA levels, and EGFR and HER2 tissue-expression in brain metastasis (BM) and overall survival (OS) in patients with advanced NSCLC. In a prospective manner, we studied 293 patients with NSCLC in IIIB-IV clinical stage. They received standard chemotherapy. CEA was measured prior to treatment; EGFR and HER2 were evaluated by immunohistochemistry. BM development was confirmed by MRI in symptomatic patients. BM developed in 27, and 32% of patients at

1 and 2 years of diagnosis with adenocarcinoma (RR 5.2; 95% CI, 1.002-29; p = 0.05) and CEA > or = 40 ng/mL (RR 11.4; 95% CI, 1.7-74; p < 0.01) as independent associated factors. EGFR and HER2 were not statistically significant. Masculine gender (RR 1.4; 95% CI, 1.002-1.9; p = 0.048), poor performance status (RR 1.8; 95% CI, 1.5-2.3; p = 0.002), advanced clinical stage (RR 1.44; 95% CI, 1.02-2; p = 0.04), CEA > or = 40 ng/mL (RR 1.5; 95% CI, 1.09-2.2; p = 0.014) and EGFR expression (RR 1.6; 95% CI, 1.4-1.9; p = 0.012) were independent associated factors to worse OS. CONCLUSION: High CEA serum level is a risk factor for BM development and is associated with poor prognosis in patients with advanced NSCLC. Surface expression of CEA in tumor cells could be the physiopathological mechanism for invasion to CNS.

Bekar, A., G. Cecener, et al. (2007). "Investigation of mutations and expression of the FHIT gene in Turkish patients with brain metastases derived from non-small cell lung cancer." Tumori **93**(6): 604-7.

Brain metastases occur in 20-40% of patients with cancer, and their frequency has increased over time. Lung, breast and skin (melanoma) are the most common sources of brain metastases. Recent studies show that several genes such as CD44 and PTEN have roles in the suppression of metastatic growth. Although it has been determined that there is a relationship between the FHIT gene and several primary tumors, its role in the initiation and progression of brain tumors has not yet been entirely explained. Furthermore, it is not known whether the FHIT gene has a role in the formation of brain metastases. The present study investigated mutations of the FHIT gene in Turkish patients with brain metastases derived from non-small cell lung cancer (NSCLC). Single-strand conformational polymorphism and sequencing analysis of the coding exons (5-9) of the FHIT gene were performed on 26 tissues. Furthermore, the level of Fhit protein expression of 36 tumor tissues was identified by immunohistochemistry. Using single-strand conformational polymorphism and sequencing analyses, no point mutations of the FHIT gene were detected in brain metastases derived from NSCLC. However, it was observed that Fhit protein expression was reduced in 88.9% of subjects. CONCLUSIONS: We suggest that the FHIT gene may be turned off in brain metastases via other genetic/epigenetic mechanisms rather than mutations.

Bogler, O. and R. Sawaya (2008). "Biomarkers and cancer stem cells in primary brain tumors. Foreword." Curr Probl Cancer **32**(3): 95-6.

Bubb, R. S., R. Komaki, et al. (2002). "Association of Ki-67, p53, and bcl-2 expression of the primary non-small-cell lung cancer lesion with brain metastatic lesion." Int J Radiat Oncol Biol Phys **53**(5): 1216-24.

The study was conducted to determine whether immunohistochemical analysis of Ki-67, p53, and bcl-2 in patients with non-small-cell lung cancer is associated with a higher rate of brain metastases and whether the intrapatient expression of these biomarkers (in the primary tumors vs. brain lesions) is similar. At the M. D. Anderson Cancer Center, tumors from 29 case patients with primary lung tumor and brain metastasis and 29 control patients with primary lung tumor but no brain metastasis were resected and examined for immunohistochemical expression. Ki-67, p53, and bcl-2 were analyzed in resected primary lung, lymph node, and metastatic brain tumors. Each control patient was matched by age, gender, and histology to a patient with brain metastasis. No significant differences in patient survival characteristics were detected between the case group and control group. Also, difference in patient outcome between the two groups was not generally predicted by biomarker analysis. However, when the groups were combined, the biomarker analysis was predictive for certain patient outcome end points. Using median values as cutoff points between low and high expression of biomarkers, it was observed that high expression of Ki-67 (>40%) in lung primaries was associated with poorer disease-free survival (p = 0.04), whereas low expression of p53 in lung primaries was associated with poorer overall survival (p = 0.04), and these patients had a higher rate of nonbrain distant metastases (p = 0.02). The patients with brain metastases were particularly prone to developing nonbrain distant metastases if the percentage of p53-positive cells in brain metastases was low (p = 0.01). There was a positive correlation in the expression of Ki-67 (p = 0.02)(r(2) = 0.1608), as well as p53 (p < 0.001) (r(2) = 0.7380), between lung primaries and brain metastases. Compared to Ki-67 and p53, bcl-2 was the least predictive. CONCLUSION: Differences in biomarker expression between the case and control groups did not serve as significant predictors of brain metastasis or patient survival. There was a strong correlation between lung primary biomarker expression and brain metastasis expression for Ki-67 and p53. Univariate analysis showed that low p53 and high Ki-67 expression predicted poor prognosis. This study shows that there may be a strong correlation between biomarker expression in non-small-cell lung cancer primary tumors and their brain metastases.

Chargari, C., Y. M. Kirova, et al. (2009). "Concurrent capecitabine and whole-brain radiotherapy for

treatment of brain metastases in breast cancer patients." J Neurooncol **93**(3): 379-84.

Preclinical data have demonstrated that ionizing radiation acts synergistically with capecitabine. This report retrospectively assessed the use of capecitabine concurrently with whole-brain radiotherapy (WBRT) in patients with brain metastases from breast cancer. From January 2003 to March 2005, five breast cancer patients with brain metastases were referred for WBRT with concurrent capecitabine. Median age was 44 years (range: 38-53). The median dose of capecitabine was 1,000 mg/m(2) twice daily for 14 days (day1-14). Treatment cycles were repeated every 21 days, concurrently with WBRT (30 Gy, 3 Gy per fraction, 5 days per week). Median survival after starting WBRT plus capecitabine was 6.5 months (range 1-34 months). One patient achieved a complete response. Two patients achieved partial response, including one with local control lasting until most recent follow-up. One patient had stable disease. The remaining patient was not assessable for response because of early death. Most commonly reported adverse events were nausea (n = 2) and headache (n = 2), always grade 1. Other toxicities were grade 3 hand/foot syndrome (n = 1), moderate anemia requiring transfusion and dose reduction of capecitabine (n = 1), and grade 1 mucositis (n = 1). Although promising, these preliminary data warrant further assessment of capecitabine-based chemoradiation in brain metastases from breast cancer and need to be further validated in the setting of a clinical trial.

Chen, E. I., J. Hewel, et al. (2007). "Adaptation of energy metabolism in breast cancer brain metastases." Cancer Res **67**(4): 1472-86.

Brain metastases are among the most feared complications in breast cancer, as no therapy exists that prevents or eliminates breast cancer spreading to the brain. New therapeutic strategies depend on specific knowledge of tumor cell properties that allow breast cancer cell growth within the brain tissue. To provide information in this direction, we established a human breast cancer cell model for brain metastasis based on circulating tumor cells from a breast cancer patient and variants of these cells derived from bone or brain lesions in immunodeficient mice. The brain-derived cells showed an increased potential for brain metastasis in vivo and exhibited a unique protein expression profile identified by large-scale proteomic analysis. This protein profile is consistent with either a selection of predisposed cells or bioenergetic adaptation of the tumor cells to the unique energy metabolism of the brain. Increased expression of enzymes involved in glycolysis, tricarboxylic acid cycle, and oxidative phosphorylation pathways suggests that the brain metastatic cells derive energy from glucose oxidation. The cells further showed enhanced activation of the pentose phosphate pathway and the glutathione system, which can minimize production of reactive oxygen species resulting from an enhanced oxidative metabolism. These changes promoted resistance of brain metastatic cells to drugs that affect the cellular redox balance. Importantly, the metabolic alterations are associated with strongly enhanced tumor cell survival and proliferation in the brain microenvironment. Thus, our data support the hypothesis that predisposition or adaptation of the tumor cell energy metabolism is a key element in breast cancer brain metastasis, and raise the possibility of targeting the functional differentiation in breast cancer brain lesions as a novel therapeutic strategy.

Chen, J. Y., J. Liu, et al. (2003). "[Suppressive effect of brain derived neurotrophic factor on H2O2-induced apoptosis in human lung cancer cell YTMLC-90]." Ai Zheng **22**(9): 938-42.

It has been shown that neurotrophic factors such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophin (NT-3/4) are synthesized in a variety of cells inside and outside the nervous system. These factors are not only able to promote neural survival, proliferation and apoptosis of neural cells but also relevant to the activity of neural invasion of tumors. It has not been reported to date whether BDNF may play roles in the biological behavior of human lung cancer cells. The aim of this experiment was to investigate the effect of BDNF on hydrogen peroxide (H2O2)-induced apoptosis in the human lung cancer cell line YTMLC-90. The minigene pSVCEPBFCAT containing the promoter and enhancer elements of the human a1(I) collagen gene(COLIA1) at its 3' terminus followed by hBDNF gene cDNA was transfected and derived BDNF ectopic expression in the human lung cancer cells. The cell proliferation was measured by MTT assay. The morphological and ultra-structural changes of apoptotic cells were observed by microscopy with fluorescent stain of acridine orange and electron microscopy. The DNA fragmentation was examined by agarose gel electrophoresis. After exposure of growing cells to 200 micromol/L H2O2 for 24 hours, the inhibition rate of cell growth was 30% in the pSVCEPBFCAT-transfected YTMLC-90, 84.60% in controls of non-transfected YTMLC-90, and 80.00% in pSVCEPCAT-transfected YTMLC-90, respectively ($P< 0.001$). The morphological and biochemical changes of apoptotic cells such as shrinkage of cytoplasm and nucleus,fragmentation of the chromatin, and ladder pattern of DNA were commonly observed in the cell population of controls, but these apoptotic features were not discovered in the

pSVCEPBFCAT-transfected YTMLC-90. CONCLUSION: BDNF markedly inhibits H2O2 cytotoxicity on human lung cancer cell YTMLC-90 by promoting YTMLC-90 proliferation and antagonizing H2O2-induced apoptosis.

Chen, J. Y., X. M. Wang, et al. (2006). "[Inhibitory effect of human brain myelin basic protein on H2O2-induced apoptosis of human lung cancer cell line YTLMC-90]." Ai Zheng **25**(2): 170-4.

Human brain myelin basic protein (MBP) distributes in nervous system and other tissues extensively, and can be detected in many kinds of tumor cells, such as lung cancer, breast cancer, and neuroglioma. However, it has not been reported whether MBP is relevant to the activity of neural invasion of tumors and whether MBP plays a role in biological behaviors of human lung cancer cells. This study was to investigate the inhibitory effect of MBP on hydrogen peroxide (H2O2)-induced apoptosis of human lung cancer cell line YTLMC-90. YTLMC-90 cells were transfected with plasmid pSVCEPMBPCAT containing MBP cDNA minigene (test group), or empty vector pSVCEPCAT, or received no transfection (control group), and exposed to H2O2. The expression of MBP in YTLMC-90 cells was detected by Western blot. Cell proliferation was measured by MTT assay. The morphologic and ultra-structural changes of apoptotic cells were observed by microscopy with fluorescent staining of acridine orange (AO) and electron microscopy. The DNA fragmentation was examined by agarose gel electrophoresis. After exposed to 200 micromol/L H2O2 for 24 h, the inhibitory rate of cell growth was significantly lower in test group than in empty vector group and control group (36.67% vs. 78.67% and 84.00%, P<0.001). The morphologic and biochemical changes of apoptotic cells, such as shrinkage of cytoplasm and nucleus, fragmentation of chromatin, and ladder pattern of DNA, were commonly observed in cells in control group, but these apoptotic features were not discovered in test group. CONCLUSION: MBP markedly inhibits H2O2 cytotoxicity to YTLMC-90 cells through promoting cell proliferation and antagonizing H2O2-induced apoptosis.

Church, D. N., A. Bahl, et al. (2006). "HER2-positive breast cancer brain metastases: multiple responses to systemic chemotherapy and trastuzumab--a case report." J Neurooncol **79**(3): 289-92.

Brain metastases from metastatic breast cancer typically occur in 10-15% of patients and are associated with survival of 3-6 months. Recent series have shown that women with HER2-positive metastatic breast cancer receiving the drug trastuzumab develop brain metastases more frequently than this, but also that continuation of trastuzumab after diagnosis of brain metastases in such patients is associated with extended survival. Authors have speculated that this is due to improved systemic control of disease; however, a possibility is that trastuzumab may have a beneficial effect on cerebral metastases themselves. We report the case of a woman with HER2-positive metastatic breast cancer who developed multiple brain metastases while on trastuzumab, in whom the addition of systemic chemotherapy to continued trastuzumab has produced multiple treatment responses associated with prolonged survival. This is the first report of its kind.

Fukayama, M., N. Funata, et al. (1990). "Brain-associated small-cell lung cancer antigen (BASCA) is expressed in developing lung: an immunohistochemical and immunoelectron microscopic study." J Histochem Cytochem **38**(1): 51-7.

Expression of brain-associated small-cell lung cancer antigen (BASCA) in developing lung and in lung tumors was investigated immunohistochemically and immunoelectron microscopically with monoclonal antibodies recognizing different epitopes of BASCA. In fetal lung, epithelial and mesenchymal cells had different spatial and temporal expression patterns, in contrast to the consistent pattern in neural cells. The cell membranes of epithelial cells of the proximal bronchial tubes were diffusely positive at the pseudoglandular stage. Ciliated cells lost immunoreactivity shortly after their emergence, but non-ciliated cells, including endocrine cells, lost it at the alveolar stage. The immunoreactivity in mesenchymal cells was reduced in the proximal airway, but positivity remained in the distal lung later during the postnatal period. All endocrine tumors of the lung, defined by diffuse synaptophysin immunoreactivity, expressed BASCA, but some non-endocrine carcinomas which also lacked densely cored granules ultrastructurally, showed BASCA positivity. The temporal and spatial pattern of BASCA expression in the developing lung suggests that BASCA plays an active role in lung morphogenesis. BASCA may be expressed as an oncofetal substance in some non-endocrine carcinomas of the lung.

Gaedcke, J., F. Traub, et al. (2007). "Predominance of the basal type and HER-2/neu type in brain metastasis from breast cancer." Mod Pathol **20**(8): 864-70.

Although breast cancer is the second most common cause of central nervous system (CNS) metastases with a notable increase of incidence, only few studies on brain-metastasizing breast cancer are available. In this immunohistochemical and

fluorescence in situ hybridization (FISH) study, metastases to the CNS (n=85) and primary breast cancers, with known involvement of the CNS (n=44) including paired primary and metastasized tumours (n=23), were investigated retrospectively for the expression of oestrogen- (ER) and progesterone- (PR) hormone receptors, Her-2/neu, epidermal growth factor receptor (EGFR), Ki-67, and cytokeratins (CKs) 5/14. The majority of brain metastases were steroid hormone receptor negative (ER 66%, PR 82%) corresponding to the findings in primary tumours with known involvement of the CNS (68% ER-negative, 75% PR-negative). The frequency of HER-2/neu-overexpressing or -amplified cancers was increased in both groups (34 and 32%, respectively). EGFR expression was more frequent in metastases (41%) than in primary tumours (16%). The proportions of cases with a basal phenotype were 26 and 30%, respectively. In paired primary tumours and metastases to the CNS, constancy of Her-2/neu status was observed in 87% of cases with only one sample turning Her-2/neu-negative and two samples acquiring overexpression/amplification in brain metastases. In contrast, steroid hormone receptors exhibited more frequently a loss of expression (17%) than a gain (9%) with 74% revealing a constant phenotype. We conclude that brain-metastasizing breast cancer belongs predominantly to the basal type or Her-2/neu type. Primary and metastatic tumours differ from each other only in a minority of cases, leading rather to a loss of steroid hormone receptors and to a gain of EGFR and Her-2/neu.

Gril, B., D. Palmieri, et al. (2008). "Effect of lapatinib on the outgrowth of metastatic breast cancer cells to the brain." J Natl Cancer Inst **100**(15): 1092-103.

The brain is increasingly being recognized as a sanctuary site for metastatic tumor cells in women with HER2-overexpressing breast cancer who receive trastuzumab therapy. There are no approved or widely accepted treatments for brain metastases other than steroids, cranial radiotherapy, and surgical resection. We examined the efficacy of lapatinib, an inhibitor of the epidermal growth factor receptor (EGFR) and HER2 kinases, for preventing the outgrowth of breast cancer cells in the brain in a mouse xenograft model of brain metastasis. EGFR-overexpressing MDA-MB-231-BR (231-BR) brain-seeking breast cancer cells were transfected with an expression vector that contained or lacked the HER2 cDNA and used to examine the effect of lapatinib on the activation (ie, phosphorylation) of cell signaling proteins by immunoblotting, on cell growth by the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, and on cell migration using a Boyden chamber assay. The outgrowth of large (ie, >50 microm(2)) and micrometastases was counted in brain sections from nude mice that had been injected into the left cardiac ventricle with 231-BR cells and, beginning 5 days later, treated by oral gavage with lapatinib or vehicle (n = 22-26 mice per treatment group). All statistical tests were two-sided. In vitro, lapatinib inhibited the phosphorylation of EGFR, HER2, and downstream signaling proteins; cell proliferation; and migration in 231-BR cells (both with and without HER2). Among mice injected with 231-BR-vector cells, those treated with 100 mg lapatinib/kg body weight had 54% fewer large metastases 24 days after starting treatment than those treated with vehicle (mean number of large metastases per brain section: 1.56 vs 3.36, difference = 1.80, 95% confidence interval [CI] = 0.92 to 2.68, P < .001), whereas treatment with 30 mg lapatinib/kg body weight had no effect. Among mice injected with 231-BR-HER2 cells, those treated with either dose of lapatinib had 50%-53% fewer large metastases than those treated with vehicle (mean number of large metastases per brain section, 30 mg/kg vs vehicle: 3.21 vs 6.83, difference = 3.62, 95% CI = 2.30 to 4.94, P < .001; 100 mg/kg vs vehicle: 3.44 vs 6.83, difference = 3.39, 95% CI = 2.08 to 4.70, P < .001). Immunohistochemical analysis revealed reduced phosphorylation of HER2 in 231-BR-HER2 cell-derived brain metastases from mice treated with the higher dose of lapatinib compared with 231-BR-HER2 cell-derived brain metastases from vehicle-treated mice (P < .001). CONCLUSIONS: Lapatinib is the first HER2-directed drug to be validated in a preclinical model for activity against brain metastases of breast cancer.

Gupta, K., J. Bishop, et al. (2004). "Antimitotic antifungal compound benomyl inhibits brain microtubule polymerization and dynamics and cancer cell proliferation at mitosis, by binding to a novel site in tubulin." Biochemistry **43**(21): 6645-55.

The antifungal agent benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate] is used throughout the world against a wide range of agricultural fungal diseases. In this paper, we investigated the interaction of benomyl with mammalian brain tubulin and microtubules. Using the hydrophobic fluorescent probe 1-anilinonaphthalene-8-sulfonic acid, benomyl was found to bind to brain tubulin with a dissociation constant of 11.9 +/- 1.2 microM. Further, benomyl bound to at a novel site, distinct from the well-characterized colchicine and vinblastine binding sites. Benomyl altered the far-UV circular dichroism spectrum of tubulin and reduced the accessibility of its cysteine residues to modification by 5,5'-dithiobis-2-nitrobenzoic acid, indicating that benomyl binding to tubulin induces a

conformational change in the tubulin. Benomyl inhibited the polymerization of brain tubulin into microtubules, with 50% inhibition occurring at a concentration of 70-75 microM. Furthermore, it strongly suppressed the dynamic instability behavior of individual brain microtubules in vitro as determined by video microscopy. It reduced the growing and shortening rates of the microtubules but did not alter the catastrophe or rescue frequencies. The unexpected potency of benomyl against mammalian microtubule polymerization and dynamics prompted us to investigate the effects of benomyl on HeLa cell proliferation and mitosis. Benomyl inhibited proliferation of the cells with an IC(50) of 5 microM, and it blocked mitotic spindle function by perturbing microtubule and chromosome organization. The greater than expected actions of benomyl on mammalian microtubules and mitosis together with its relatively low toxicity suggest that it might be useful as an adjuvant in cancer chemotherapy.

Hayashi, K., K. Yamauchi, et al. (2009). "A color-coded orthotopic nude-mouse treatment model of brain-metastatic paralyzing spinal cord cancer that induces angiogenesis and neurogenesis." Cell Prolif **42**(1): 75-82.

Cancer of the spinal cord is highly malignant and often leads to paralysis and death. A realistic mouse model would be an important benefit for the better understanding and treatment of spinal cord glioma. To develop an imageable, patient-like model of this disease, U87 human glioma tumour fragments (expressing red fluorescent protein), were transplanted by surgical orthotopic implantation into the spinal cord of nontransgenic nude mice or transgenic nude mice expressing nestin-driven green fluorescent protein (ND-GFP). In ND-GFP mice, GFP is expressed in nascent blood vessels and neural stem cells. The animals were treated with temozolomide or vehicle control. The intramedullary spinal cord tumour grew at the primary site, caused hind-limb paralysis and also metastasized to the brain. Temozolomide inhibited tumour growth ($P<0.01$) and prevented metastasis, as well as prevented paralysis in four mice and delayed paralysis in two mice of the six tested ($P=0.005$). In the ND-GFP-expressing host, ND-GFP cells staining positively for neuronal class III-beta-tubulin or CD31, surrounded the tumour. These results suggest that the tumour stimulated both neurogenesis and angiogenesis, respectively. CONCLUSION: A patient-like model of spinal cord glioma was thus developed, which can be used for the discovery of new agents, including those that inhibit invasion and metastasis of the disease as well as those that prevent paralysis.

He, B. P., J. J. Wang, et al. (2006). "Differential reactions of microglia to brain metastasis of lung cancer." Mol Med **12**(7-8): 161-70.

The brain is a common metastatic site for various types of cancers, especially lung cancer. Patients with brain metastases have a poor prognosis in spite of radiotherapy and/or chemotherapy. It is postulated that immune cells in the brain may play a major role in cancer metastasis, dormancy, and relapse. Although microglia may serve as a major component in the brain immune system, the interaction between metastatic cancer cells and microglia is still largely unknown and remains to be elucidated. In this study, we have investigated microglial reactions in brain tissues with metastatic lung cancer cells and evaluated the cytotoxic effects of lipopolysaccharide (LPS)-activated microglia on metastatic lung cancer cells in vitro. In the vicinity of metastatic lung cancer mass in the brain, microglia showed signs of significant activation. There was an obvious increase in the number of microglia labeled with ionized calcium binding adaptor molecule 1 (Iba-1) antibody, a specific marker of microglia. The microglia were observed to form a clear boundary between the tumor mass and normal brain tissue. In the region where the tumor mass was situated, only a few microglia expressed inducible nitric oxide synthase (iNOS) and tumor necrosis factor-alpha (TNF-alpha), indicating differential activation in those microglia. The supernatant from LPS-activated microglia induced apoptosis of metastatic lung cancer cells in vitro in a dose- and time-dependent manner. However, at lower concentrations of activated microglial supernatant, trophic effects on cancer cells were observed, some lung cancer cells being insensitive to microglial cytotoxicity. Together with the observation that TNF-alpha alone induced proliferation of the tumor cells, the findings provide possible clues to the mechanism involved in metastasis of lung cancer cells to the brain.

Hines, S. L., L. A. Vallow, et al. (2008). "Clinical outcomes after a diagnosis of brain metastases in patients with estrogen- and/or human epidermal growth factor receptor 2-positive versus triple-negative breast cancer." Ann Oncol **19**(9): 1561-5.

Women with triple-negative (TN) breast cancer are at increased risk of distant metastases and have reduced survival versus other breast cancer patients. Relative survival of women with TN breast cancer who develop brain metastases is unknown. Patients with breast cancer who developed brain metastases at our institution from 1993 to 2006 were reviewed. Four survival time intervals were compared in patients with TN disease and those with non-TN disease: initial diagnosis to distant metastases, distant

metastases to brain metastases, brain metastases to death, and overall diagnosis to death. One hundred and eighteen patients were identified. Fifty-one (50%) of 103 were estrogen receptor positive, 26 (39%) of 67 were human epidermal growth factor receptor 2 positive, and 20 (22%) of 91 were TN. Survival times were shorter for TN patients, with overall survival of 26 months in TN patients versus 49 months for non-TN patients. In TN patients, time to development of distant metastases, brain metastases, and death after brain metastases was shorter than in non-TN patients. CONCLUSION: Patients with TN disease were more likely to develop distant metastases earlier than non-TN patients, developed brain metastases sooner, and had shorter overall survival.

Jacobs, J. F., O. M. Grauer, et al. (2008). "Selective cancer-germline gene expression in pediatric brain tumors." J Neurooncol **88**(3): 273-80.

Cancer-germline genes (CGGs) code for immunogenic antigens that are present in various human tumors and can be targeted by immunotherapy. Their expression has been studied in a wide range of human tumors in adults. We measured the expression of 12 CGGs in pediatric brain tumors, to identify targets for therapeutic cancer vaccines. Real Time PCR was used to quantify the expression of genes MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, MAGE-A12, MAGE-C2, NY-ESO-1 and GAGE-1,2,8 in 50 pediatric brain tumors of different histological subtypes. Protein expression was examined with immunohistochemistry. Fifty-five percent of the medulloblastomas (n = 11), 86% of the ependymomas (n = 7), 40% of the choroid plexus tumors (n = 5) and 67% of astrocytic tumors (n = 27) expressed one or more CGGs. Immunohistochemical analysis confirmed qPCR results. With exception of a minority of tumors, the overall level of CGG expression in pediatric brain tumors was low. We observed a high expression of at least one CGG in 32% of the samples. CGG-encoded antigens are therefore suitable targets in a very selected group of pediatric patients with a brain tumor. Interestingly, glioblastomas from adult patients expressed CGGs more often and at significantly higher levels compared to pediatric glioblastomas. This observation is in line with the notion that pediatric and adult glioblastomas develop along different genetic pathways.

Khalil, A. A. (2007). "Biomarker discovery: a proteomic approach for brain cancer profiling." Cancer Sci **98**(2): 201-13.

Gliomas in the form of astrocytomas, anaplastic astrocytomas and glioblastomas are the most common brain tumors in humans. Early detection of these cancers is crucial for successful treatment. Proteomics promises the discovery of biomarkers and tumor markers for early detection and diagnosis. In the current study, a differential gel electrophoresis technology coupled with matrix-assisted laser desorption/ionization-time of flight and liquid chromatography-tandem mass spectroscopy was used to investigate tumor-specific changes in the proteome of human brain cancer. Fifty human brain tissues comprising varying diagnostic groups (non-tumor, grade I, grade II, grade III and grade IV) were run in duplicate together with an internal pool sample on each gel. The proteins of interest were automatically picked, in-gel digested and mass spectrometry fingerprinted. Two hundred and eleven protein spots were identified successfully and were collapsed into 91 unique proteins. Approximately 20 of those 91 unique proteins had, to our knowledge, not been reported previously as differentially expressed in human brain cancer. Alb protein, peroxiredoxin 4 and SH3 domain-binding glutamic acid-rich-like protein 3 were upregulated in glioblastoma multiform versus non-tumor tissues. However, aldolase C fructose-biphosphate, creatine kinase, B chain dihydrolipoyl dehydrogenase, enolase 2, fumarate hydratase, HSP60, lactoylglutathione lyase, lucine aminopeptidase, Mu-crystallin homolog, NADH-UO 24, neurofilament triplet L protein, septin 2, stathmin and vacuolar ATP synthase subunit E were downregulated in glioblastoma multiform compared with non-tumor tissues. These differentially expressed proteins provided novel information on the differences existing between normal brain and gliomas, and thus might prove to be useful molecular indicators of diagnostic or prognostic value.

Kim, Y. J. and W. Feiden (2007). "A 76-year-old woman with a history of bladder cancer and new brain lesion." Brain Pathol **17**(3): 329-30.

Leszczynski, D., S. Joenvaara, et al. (2002). "Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and blood-brain barrier-related effects." Differentiation **70**(2-3): 120-9.

We have examined whether non-thermal exposures of cultures of the human endothelial cell line EA.hy926 to 900 MHz GSM mobile phone microwave radiation could activate stress response. Results obtained demonstrate that 1-hour non-thermal exposure of EA.hy926 cells changes the phosphorylation status of numerous, yet largely unidentified, proteins. One of the affected proteins was identified as heat shock protein-27 (hsp27). Mobile phone exposure caused a transient increase in

phosphorylation of hsp27, an effect which was prevented by SB203580, a specific inhibitor of p38 mitogen-activated protein kinase (p38MAPK). Also, mobile phone exposure caused transient changes in the protein expression levels of hsp27 and p38MAPK. All these changes were non-thermal effects because, as determined using temperature probes, irradiation did not alter the temperature of cell cultures, which remained throughout the irradiation period at 37 +/- 0.3 degrees C. Changes in the overall pattern of protein phosphorylation suggest that mobile phone radiation activates a variety of cellular signal transduction pathways, among them the hsp27/p38MAPK stress response pathway. Based on the known functions of hsp27, we put forward the hypothesis that mobile phone radiation-induced activation of hsp27 may (i) facilitate the development of brain cancer by inhibiting the cytochrome c/caspase-3 apoptotic pathway and (ii) cause an increase in blood-brain barrier permeability through stabilization of endothelial cell stress fibers. We postulate that these events, when occurring repeatedly over a long period of time, might become a health hazard because of the possible accumulation of brain tissue damage. Furthermore, our hypothesis suggests that other brain damaging factors may co-participate in mobile phone radiation-induced effects.

Mayoral, M. A., C. Mayoral, et al. (2008). "Identification of galectin-3 and mucin-type O-glycans in breast cancer and its metastasis to brain." Cancer Invest **26**(6): 615-23.

Galectin-3 has been implicated in tumor progression. We demonstrated immunohistochemically that galectin-3 was negative in normal breast tissue, but it was highly increased in breast cancer and in metastatic tissues to brain. Similarly, histochemistry with mucin-specific lectins showed increased recognition in breast tumor and metastasis with Machaerocereus eruca agglutinin (Fualpha 1,2 (GalNAcalpha 1,3) Galss1,4 in complex mucin) but not for Amaranthus leucocarpus (Galss1,3-GalNAc-alpha 1,0-Ser/Thr) and Arachis hypogaea lectins (Galss1,3GalNAc/Galss1,4GlcNAc). Mucin-type glycans and galectin-3 colocalized in breast cancer and metastasis, but not in normal tissue, suggesting upregulated biosynthesis of complex O-glycosidically linked glycans and galectin-3 favor breast cancer progression and brain metastasis.

Mendes, O., H. T. Kim, et al. (2005). "Expression of MMP2, MMP9 and MMP3 in breast cancer brain metastasis in a rat model." Clin Exp Metastasis **22**(3): 237-46.

In order to study the expression of MMP2, MMP3 and MMP9 in breast cancer brain metastasis, we used a syngeneic rat model of distant metastasis of ENU1564, a carcinogen-induced mammary adenocarcinoma cell line. At six weeks post inoculation we observed development of micro-metastasis in the brain. Immunohistochemistry and Western Blotting analyses showed that MMP-2, -3 and -9 proteins expressions are consistently significantly higher in neoplastic brain tissue compared to normal brain tissue. These results were confirmed by RT-PCR. In situ zymography revealed gelatinase activity within the brain metastasis. Gel zymography showed increase in MMP2 and MMP3 activity in brain metastasis. Furthermore, we were able to significantly decrease the development of breast cancer brain metastasis in animals by treatment with PD 166793, a selective synthetic MMP inhibitor. In addition, PD 166793 decreased the in vitro invasive cell behavior of ENU1546. Together our results suggest that MMP-2, -3 and -9 may be involved in the process of metastasis of breast cancer to the brain.

Milas, I., R. Komaki, et al. (2003). "Epidermal growth factor receptor, cyclooxygenase-2, and BAX expression in the primary non-small cell lung cancer and brain metastases." Clin Cancer Res **9**(3): 1070-6.

The purpose is to identify biological markers that predict brain metastasis and treatment outcome in non-small cell lung cancer (NSCLC). EXPERIMENTAL DESIGN: Samples were obtained from the primary tumors, lymph nodes, and brain metastases of 29 patients with NSCLC who had undergone resection of both the pulmonary tumors and the brain lesions. Samples from 29 patients matched for age, sex, and histology whose pulmonary tumors were resected served as controls. Samples were stained with H&E as well as immunohistochemical stains for epidermal growth factor receptor (EGFR), cyclooxygenase 2 (COX-2), and BAX. Comparisons were made between patients with and without brain metastasis. Independent investigators determined the percentage of positive cells. There was positive correlation in expression of all three biomarkers between primary lung tumors and lymph node metastases. Significantly higher levels of EGFR were found in lymph node metastases in the control group (P = 0.0147). COX-2 expression in brain lesions correlated with expression in primary tumors (P = 0.023). BAX levels were lower in poorly differentiated tumors in lymph node metastases in the control group (P = 0.01) and in brain metastases (P = 0.045). Low EGFR expression and high COX-2 expression in lymph node metastasis were associated with poorer treatment outcome. CONCLUSIONS: Expression of EGFR, COX-2, and BAX in primary lung tumors did not differ between patients with brain metastases from NSCLC and those without brain

metastases. These three biomarkers cannot be used to predict brain metastasis. Studies of other biomarkers are under way in an effort to predict brain metastasis among patients with NSCLC.

Mravec, B., L. Lackovicova, et al. (2009). "Brain response to induced peripheral cancer development in rats: dual fos-tyrosine hydroxylase and fos-oxytocin immunohistochemistry." Endocr Regul **43**(1): 3-11.

During last few decades a considerable number of data has emerged supporting the hypothesis that central nervous system might monitor and modulate tumor growth. This assumption is based on two facts: 1. immune system plays a crucial role in the development and progression of cancer; 2. immune and nervous systems communicate tightly and bidirectionally. The aim of present study was to elucidate whether tumor growth may induce detectable changes in brain structures that are involved in the response to immune challenges. Using Fos immunohistochemistry, we investigated whether the advanced stage of cancer, induced by a single intraperitoneal injection of BP6-TU2 fibrosarcoma cells to male Wistar rats, could activate Fos expression in the nucleus of the solitary tract (NTS), amygdala and parabrachial nuclei (PBN) and also activate some of neuronal phenotypes including tyrosine hydroxylase (TH) neurons in the brainstem noradrenergic cell groups and hypothalamic oxytocinergic neurons. Twenty eight days after the initiation of tumor process we found increased Fos expression in NTS/A2, A1 noradrenergic cells, PBN as well as in the hypothalamic paraventricular, supraoptic and accessory oxytocinergic neurons. These structures are involved in the transmission of signals related to immune challenges within the brain and consequent elaboration of neuro-endocrine responses. CONCLUSIONS: The data obtained are supporting the view that the information on peripheral tumor development might be transmitted to the brain. However, further studies are necessary to be performed to reveal whether our findings can be attributed to specific effect of cancer or whether observed changes in the activity of brainstem and hypothalamic neurons reflex processes that only accompany the cancer progression.

Ogawa, M., K. Kurahashi, et al. (2007). "Miliary brain metastasis presenting with dementia: progression pattern of cancer metastases in the cerebral cortex." Neuropathology **27**(4): 390-5.

We report an autopsy case of an 82-year-old woman with progressive dementia due to miliary brain metastasis from lung adenocarcinoma. The patient presented with dementia 5 months prior to death and suddenly died of pulmonary hemorrhage. Postmortem examination revealed normal appearance of the brain. However, there were numerous foci of cancer metastasis in all parts of the brain on light microscopic examination. The carcinoma cells were located in the perivascular (Virchow-Robin) space and did not invade to the brain parenchyma. The carcinoma cells were also found in the subpial space. In the cerebral cortex, foci of metastasis appeared to spread in the following way: tiny foci of metastasis initially occur in the middle cortical layer, then spread to all layers through the perivascular space, and finally reach the subpial space and subcortical white matter. Although the junction between gray and white matter is a preferred site for usual brain metastasis, middle cortical layer was considered to be the initial site for metastasis in our patient. The perivascular pial sheath plays an important role for the development of miliary brain metastasis.

Onodera, H., S. Nagayama, et al. (2005). "Brain metastasis from colorectal cancer." Int J Colorectal Dis **20**(1): 57-61.

The mechanism of brain metastasis is not well understood, but the affinity between cancer cells and neural tissues may be involved in the process. The aim of our study is to elucidate the involvement of neural cell adhesion molecule (NCAM) and therapeutic parameters in patients with brain metastasis from colorectal cancer. We retrospectively identified 17 patients with brain metastasis from colorectal cancer. Data were collected with regard to patients' characteristics, location, and stage of primary tumor, and extent and location of metastatic disease. NCAM histochemical staining was undertaken using a paraffin block, and compared with 56 Dukes C patients and 13 Dukes D patients. Neural cell adhesion molecule expression was significantly higher in the primary tumors of the brain metastasis patients than in the lesions of the Dukes C and Dukes D control groups ($p = 0.0004$). Patients whose tumor was managed by radiosurgery survived longer than patients who had had whole brain radiation or those who had been left untreated. CONCLUSION: The fact that NCAM expression was high in the primary tumors of brain metastasis patients suggests that the affinity of cancer cells to a particular organ is important for circulation-mediated metastasis. Controlling local tumors using radiosurgery is certainly going to play an important role in extending survival and improving the patient's quality of life (QOL).

Perry, J. J., L. Fan, et al. (2007). "Developing master keys to brain pathology, cancer and aging from the structural biology of proteins controlling reactive

oxygen species and DNA repair." Neuroscience **145**(4): 1280-99.

This review is focused on proteins with key roles in pathways controlling either reactive oxygen species or DNA damage responses, both of which are essential for preserving the nervous system. An imbalance of reactive oxygen species or inappropriate DNA damage response likely causes mutational or cytotoxic outcomes, which may lead to cancer and/or aging phenotypes. Moreover, individuals with hereditary disorders in proteins of these cellular pathways have significant neurological abnormalities. Mutations in a superoxide dismutase, which removes oxygen free radicals, may cause the neurodegenerative disease amyotrophic lateral sclerosis. Additionally, DNA repair disorders that affect the brain to various extents include ataxia-telangiectasia-like disorder, Cockayne syndrome or Werner syndrome. Here, we highlight recent advances gained through structural biochemistry studies on enzymes linked to these disorders and other related enzymes acting within the same cellular pathways. We describe the current understanding of how these vital proteins coordinate chemical steps and integrate cellular signaling and response events. Significantly, these structural studies may provide a set of master keys to developing a unified understanding of the survival mechanisms utilized after insults by reactive oxygen species and genotoxic agents, and also provide a basis for developing an informed intervention in brain tumor and neurodegenerative disease progression.

Preusser, M., H. Heinzl, et al. (2006). "Histopathologic assessment of hot-spot microvessel density and vascular patterns in glioblastoma: Poor observer agreement limits clinical utility as prognostic factors: a translational research project of the European Organization for Research and Treatment of Cancer Brain Tumor Group." Cancer **107**(1): 162-70.

Hot-spot microvessel density (MVD) and vascular patterns have been reported as histopathologic factors that influence prognosis in retrospective series of malignant gliomas. To investigate clinical utility, the authors systematically studied observer agreement on MVD and vascular patterns and the influence of repeatedly assessed data on patient outcomes in 2 independent glioblastoma series. MVD and vascular patterns were assessed retrospectively by 5 observers in 1) a retrospectively compiled glioblastoma series that included 110 patients and 2) a glioblastoma series that included 233 patients who were treated within a randomized trial. MVD was determined in the field of greatest density ("hot-spot"). Predominantly classic or bizarre vascular patterns were determined by using a previously defined algorithm. Observer agreement on MVD was highly variable (range of kappa values, 0.464-0.901). The worst observer agreement was achieved when both the selection of hot-spots and MVD counts were performed independently. Survival analysis did not show a consistent association between repeatedly assessed MVD and patient outcome. Observer agreement on vascular patterns was poor (kappa = 0.297). Survival analysis did not show a consistent association between repeatedly assessed vascular patterns and patient outcome. CONCLUSIONS: Observer agreement on hot-spot MVD and vascular patterns in patients with glioblastoma was poor in independent assessments. MVD and vascular patterns were not associated consistently with patient outcome. Based on these findings, the authors concluded that poor observer agreement limits the clinical utility of histopathologically assessed hot-spot MVD and vascular patterns as prognostic factors in patients with glioblastoma. Improved methodologies for morphologic assessment of glioblastoma vascularization need to be identified.

Rzepko, R., E. Izycka-Swieszewska, et al. (1999). "The metastases of lung cancer to the brain--the examination of angiogenesis and p53 expression." Folia Neuropathol **37**(3): 195-8.

The aim of the present study was to investigate the intensity of angiogenesis and p53 protein expression in metastases of lung cancer to the brain. There were eight cases of squamous cell type and nine adenocarcinomas among 17 examined cases of metastatic carcinomas. The antibodies against von Willebrand factor (vWF)--to highlight the microvessels and against p53 protein--for detection of immunopositive cells were used. The intensity of angiogenesis was represented by the mean number of the blood vessels in three tumor fields with the highest microvascular density ("hot spots"). The measurements were taken in three microscopic fields under 200x magnification (the examined area was 0.785 mm2). The mean number of p53-positive cells in three tumor areas under 200x magnification with the highest number of p53-positive cells was the measure of protein p53 expression. The values of vascular density and p53 expression differed a lot among the examined tumors. The values of vascular density were between 4.2-106 vessels/mm2 (mean value 49.3 vessel/mm2). The number of p53-immunopositive cells was between 0-284 cells/mm2 (mean value 110.6 cells/mm2). There was no significant correlation between examined parameters (correlation coefficient 0.18).

Sahin, U., M. Koslowski, et al. (2000). "Expression of cancer testis genes in human brain tumors." Clin Cancer Res **6**(10): 3916-22.

Cancer-testis (CT) genes are expressed in a variety of human cancers but not in normal tissues, except for testis tissue, and represent promising targets for immunotherapeutic and gene therapeutic approaches. Because little is known about their composite expression in human brain tumors, we investigated the expression of seven CT genes (MAGE-3, NY-ESO-1, HOM-MEL-40/SSX-2, SSX-1, SSX-4,HOM-TES-14/SCP-1, and HOM-TES-85) in 88 human brain tumor specimens. Meningiomas expressed only HOM-TES-14/SCP-1 (18% of meningiomas were HOM-TES-14/SCP-1 positive) and did not express any other CT genes. One ependymoma was negative for all CT genes tested. SSX-4 was the only CT gene expressed in oligodendrogliomas (2 of 5 cases), and it was also expressed in oligoastrocytomas (3 of 4 cases) and astrocytomas (10 of 37 cases). Astrocytomas were most frequently positive for HOM-TES-14/SCP-1 (40%) and SSX-4 (27%), followed by HOM-TES-85 (13%), SSX-2 (11%), and MAGE-3 (7%). Whereas MAGE-3 was detected only in grade IV astrocytomas, the expression of the other CT genes showed no clear correlation with histological grade. Of 39 astrocytomas, 60% expressed at least one CT gene, 21% expressed two CT genes, and 8% coexpressed three CT genes of the seven CT genes investigated. We conclude that a majority of oligoastrocytomas and astrocytomas might be amenable to specific immunotherapeutic interventions. However, the identification of additional tu-mor-specific antigens with a frequent expression in gliomas is warranted to allow for the development of widely applicable polyvalent glioma vaccines.

Shai, R. M., J. K. Reichardt, et al. (2008). "Pharmacogenomics of brain cancer and personalized medicine in malignant gliomas." Future Oncol **4**(4): 525-34.

The pharmacogenetics of cancer treatment has been aimed at identifying genetic components of interindividual variability in patients' response to cancer chemotherapy and toxicity. This, in turn, will establish an individually based treatment, and also elucidate the molecular basis of the treatment regimen for further improvements. Brain cancer is an instructive example for the potential contributions of pharmacogenomics to improved treatment in the 21st century. Patients with oligodendrogliomas have benefited from phamacogenomics, as there is a clear relationship between response to chemotherapy and chromosomal profile. Drug efficacy, safety and response could be improved by using pharmacogenomics to identify genetic markers that differentiate responder from nonresponder patient groups, as well as identifying patients likely to develop adverse drug reactions. This review will focus on how pharmacogenomics by microarray studies may lead to much more accurate tumor classification, drug and biomarker discovery, and drug efficacy testing. We will discuss relevant scientific advances in pharmacogenetics for more personalized chemotherapy.

Singh, S. K., I. D. Clarke, et al. (2003). "Identification of a cancer stem cell in human brain tumors." Cancer Res **63**(18): 5821-8.

Most current research on human brain tumors is focused on the molecular and cellular analysis of the bulk tumor mass. However, there is overwhelming evidence in some malignancies that the tumor clone is heterogeneous with respect to proliferation and differentiation. In human leukemia, the tumor clone is organized as a hierarchy that originates from rare leukemic stem cells that possess extensive proliferative and self-renewal potential, and are responsible for maintaining the tumor clone. We report here the identification and purification of a cancer stem cell from human brain tumors of different phenotypes that possesses a marked capacity for proliferation, self-renewal, and differentiation. The increased self-renewal capacity of the brain tumor stem cell (BTSC) was highest from the most aggressive clinical samples of medulloblastoma compared with low-grade gliomas. The BTSC was exclusively isolated with the cell fraction expressing the neural stem cell surface marker CD133. These CD133+ cells could differentiate in culture into tumor cells that phenotypically resembled the tumor from the patient. The identification of a BTSC provides a powerful tool to investigate the tumorigenic process in the central nervous system and to develop therapies targeted to the BTSC.

Soling, A., M. Sackewitz, et al. (2005). "Minichromosome maintenance protein 3 elicits a cancer-restricted immune response in patients with brain malignancies and is a strong independent predictor of survival in patients with anaplastic astrocytoma." Clin Cancer Res **11**(1): 249-58.

The identification of new molecular markers in astrocytic tumors may help to understand the biology of these tumors in more detail. Informative tumor markers may represent prognostic factors for response to therapy and outcome as well as potential targets for novel anticancer therapies. EXPERIMENTAL DESIGN: Tumor-associated antigens were identified by immunoscreening of a human glioma cDNA expression library with allogeneic sera from patients with diffuse astrocytoma (WHO grades 2-4). The expression of one of the identified antigens, the replication licensing factor

minichromosome maintenance protein 3 (MCM3), was analyzed by immunohistochemistry in 142 primary and 27 recurrent astrocytomas (WHO grades 2-4). In addition, 98 serum specimens from patients with primary and secondary brain malignancies and 30 serum specimens from healthy controls were examined by serologic immunoscreening for immunoreactivity with MCM3. MCM3 is overexpressed in human astrocytic tumors and elicits a cancer-restricted humoral immune response in 9.3% (9 of 97) of patients with brain tumors (n = 95) and brain metastases (n = 2) but not in healthy controls. Expression of MCM3 in diffuse astrocytoma is significantly associated with age ($P < 0.001$), histologic grade ($P < 0.001$), time to recurrence ($P = 0.01$), and expression of the proliferation marker Ki-67 ($P < 0.001$) but not with sex ($P = 0.800$). Univariate and multivariate Cox regression analysis confirmed MCM3 expression as an independent predictor of poor outcome in astrocytoma patients ($P < 0.001$ for both). CONCLUSIONS: MCM3 may represent a glioma-associated antigen with significant prognostic role as well as have some potential as a target for cancer-directed therapy.

Stark, A. M., H. H. Hugo, et al. (2007). "p53, BCL-2 and BAX in non-small cell lung cancer brain metastases: a comparison of real-time RT-PCR, ELISA and immunohistochemical techniques." Neurol Res **29**(5): 435-40.

OBJECTIVES: Metastasis to the brain is a severe and common complication in non-small cell lung cancer (NSCLC). The examination of cell cycle associated genes in these lesions may contribute to the understanding of metastatic growths in the central nervous system. The aim of this study was to evaluate the p53, BCL-2 and BAX mRNA and protein expression in NSCLC brain metastases in comparison with matched primary tumors. For quantitative TaqMan real-time reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA), fresh frozen tumor specimens from 12 patients with NSCLC brain metastases were available. For immunohistochemical staining, 78 surgically removed NSCLC brain metastases were used. PCR results were analysed using the DeltaDeltaCT method. Staining was analysed using a modified immunoreactive score (IRS). Overall, p53, BCL-2 and BAX expression values in brain metastases and primary tumors showed a wide variety. The comparison of different techniques revealed different findings on the mRNA and protein level. Herein, PCR and ELISA revealed no clear tendencies. In contrast, immunohistochemistry showed significant overexpression of BAX and underexpression of BCL-2 in brain metastases. CONCLUSION: A high variability in the expression of p53, BCL-2 and BAX in NSCLC exists in brain metastases. Immunohistochemistry revealed overexpression of BAX and underexpression of BCL-2 in brain metastases, whereas there were no clear tendencies using PCR and ELISA techniques. More insights into the BAX/BCL-2 interaction are needed before reasonable conclusions can be drawn from the existing data.

Stark, A. M., S. Pfannenschmidt, et al. (2006). "Reduced mRNA and protein expression of BCL-2 versus decreased mRNA and increased protein expression of BAX in breast cancer brain metastases: a real-time PCR and immunohistochemical evaluation." Neurol Res **28**(8): 787-93.

OBJECTIVES: Brain metastases are an increasingly common complication in breast cancer patients. Apoptosis regulating genes are promising candidates for further treatment options. We examined the mRNA and protein expression of p53, BCL-2 and BAX in breast cancer brain metastases versus primary tumors. In a two-step approach p53, BCL-2 and BAX mRNA expression in ductal invasive breast cancer brain metastases was examined by: (1) reverse transcription-polymerase chain reaction (RT-PCR) mRNA expression screening (band appearance in relation to an internal standard) and (2) quantitative real-time RT-PCR (CT-values in relation to an internal standard). Protein expression using immunohistochemistry. Results were compared with primary tumors. We found significantly lower BCL-2 mRNA and protein expression in breast cancer brain metastases versus primary tumors. P53 mRNA and protein expression was also lower in metastases. However, this difference was only significant on mRNA but not on the protein level. BAX expression evaluation revealed was contradictory results: mRNA expression was significantly lower whereas protein expression was significantly higher in metastatic lesions. DISCUSSION: The mRNA and protein expression of p53 and BCL-2 seems to be reduced in breast cancer brain metastases. BAX mRNA and protein may be regulated differentially in metastatic lesions.

Stark, A. M., K. Tongers, et al. (2005). "Reduced metastasis-suppressor gene mRNA-expression in breast cancer brain metastases." J Cancer Res Clin Oncol **131**(3): 191-8.

Brain metastases are an increasingly common complication in breast cancer patients. The Metastasis Suppressor Genes (MSG) Nm23, KISS1, KAI1, BRMS1, and Mkk4 have been associated with the metastatic potential of breast cancer in vitro and in vivo. The mRNA expression of Nm23, KISS1, KAI1,

BRMS1, and Mkk4 in fresh frozen tissue samples of brain metastases from ductal invasive breast cancer specimens was examined in relation to primary tumors. In a first step, mRNA expression screening was carried out using a semi-quantitative RT-PCR approach, in a second step quantitative real-time RT-PCR was performed on selected specimens. By immunohistochemical staining, gene products were visualized on the protein level. Semi-quantitative RT-PCR revealed reduced mRNA expression of Nm23, KISS1, KAI1, BRMS, and Mkk4 in brain metastases. Results for KISS1, KAI1, BRMS, and Mkk4 were confirmed by real-time RT-PCR. In detail, mRNA expression reduction in breast cancer brain metastases was tenfold. Expression of MSG could be confirmed by immunohistochemical staining on protein level. CONCLUSIONS: Our investigations revealed significantly reduced mRNA expression of metastases suppressor genes KISS1, KAI1, BRMS1, and Mkk4 in breast cancer brain metastasis. Particularly, in the case of KISS1 and Mkk4, an important role for future treatment of patients with breast cancer brain metastatic lesions can be assumed.

Stemmler, H. J., S. Kahlert, et al. (2006). "Characteristics of patients with brain metastases receiving trastuzumab for HER2 overexpressing metastatic breast cancer." Breast **15**(2): 219-25.

The intention of this retrospective analysis was to describe the characteristics of patients with brain metastasis (BM) receiving trastuzumab for HER2 overexpressing metastatic breast cancer (MBC). A specific focus was the relation of BM occurrence to remission status of visceral disease during trastuzumab treatment. Patients with MBC presenting between March 2000 and May 2004 were included in this retrospective analysis. HER2 overexpression was determined by immunohistochemistry (IHC; DAKO Hercep Test). Trastuzumab was applied at a loading dose of 4 mg/kg and a maintenance dose of 2 mg/kg. Among 136 HER2 overexpressing patients (DAKO score 3+), 42 patients with BM were identified during follow-up (30.9%). Negative hormone receptor expression (estrogen receptor (ER) and progesterone receptor (PgR)) correlated with incidence of BM (42.8% vs. 23.4%; P=0.01). There was no correlation of the development of BM with regard to tumor grading and patient age. In patients who developed BM, the median interval between visceral and brain metastasis was 14 months (range 0-69 months). At the time BM was diagnosed, 14 out of 42 patients responded to trastuzumab-based treatment schedules (OR: 33.3%, 95% CI 18.5-48.2%). Median survival from diagnosis of BM was 13 months (range 0-60 months). The median overall survival calculated from first diagnosis of metastasis was not significantly shorter in patients with BM than in patients without BM (37 vs. 47 months; P=0.07 log rank). Trastuzumab is highly effective for the treatment of liver and lung metastasis in HER2 overexpressing patients, while it is apparently ineffective for treating or preventing BM. Since one third of HER2 overexpressing patients with MBC developed BM despite effective trastuzumab treatment, new treatment strategies and closer surveillance may be warranted for these patients.

Tolle, A., M. Jung, et al. (2009). "Brain-type and liver-type fatty acid-binding proteins: new tumor markers for renal cancer?" BMC Cancer **9**: 248.

Renal cell carcinoma (RCC) is the most common renal neoplasm. Cancer tissue is often characterized by altered energy regulation. Fatty acid-binding proteins (FABP) are involved in the intracellular transport of fatty acids (FA). We examined the level of brain-type (B) and liver-type (L) FABP mRNA and the protein expression profiles of both FABPs in renal cell carcinoma. Paired tissue samples of cancerous and noncancerous kidney parts were investigated. Quantitative RT-PCR, immunohistochemistry and western blotting were used to determine B- and L-FABP in tumor and normal tissues. The tissue microarray (TMA) contained 272 clinico-pathologically characterized renal cell carcinomas of the clear cell, papillary and chromophobe subtype. SPSS 17.0 was used to apply crosstables (chi2-test), correlations and survival analyses. B-FABP mRNA was significantly up-regulated in renal cell carcinoma. In normal tissue B-FABP mRNA was very low or often not detectable. RCC with a high tumor grading (G3 + G4) showed significantly lower B-FABP mRNA compared with those with a low grading (G1 + G2). Western blotting analysis detected B-FABP in 78% of the cases with a very strong band but in the corresponding normal tissue it was weak or not detectable. L-FABP showed an inverse relationship for mRNA quantification and western blotting. A strong B-FABP staining was present in 52% of the tumor tissues contained in the TMA. In normal renal tissue, L-FABP showed a moderate to strong immunoreactivity in proximal tubuli. L-FABP was expressed at lower rates compared with the normal tissues in 30.5% of all tumors. There was no correlation between patient survival times and the staining intensity of both FABPs. CONCLUSION: While B-FABP is over expressed in renal cell carcinoma in comparison to normal renal tissues L-FABP appears to be reduced in tumor tissue. Although the expression behavior was not related to the survival outcome of the RCC patients, it can be assumed that these changes indicate fundamental alterations in the fatty metabolism in the

RCC carcinogenesis. Further studies should identify the role of both FABPs in carcinogenesis, progression and with regard to a potential target in RCC.

Wang, W., E. Svanberg, et al. (2005). "NOS isoenzyme content in brain nuclei as related to food intake in experimental cancer cachexia." Brain Res Mol Brain Res **134**(2): 205-14.

Evidence implies that nitric oxide (NO) in the central nervous systems mediates anorexia in tumor-bearing hosts. We have therefore evaluated, by immunohistochemical image analyses, net alterations of nitric oxide synthases (nNOS, eNOS, iNOS) in brain nuclei [paraventricular hypothalamic nucleus (PVN), medial habenular nucleus (MHB), lateral habenular nucleus (LHB), paraventricular thalamic nucleus (PV), lateral hypothalamic area (LHA), ventromedial hypothalamic nucleus (VMH), nucleus of the solitary tract (NTS)] of tumor-bearing mice (TB) with prostanoid-related anorexia. Pair-fed (PF) and freely fed (FF) non-tumor-bearing mice were used as controls. c-fos was analyzed as indicator of neuronal activation. nNOS was significantly increased in VMH and PVN from TB mice, while eNOS was significantly increased in LHB and LHA. iNOS was significantly increased in LHA and PVN nuclei, but decreased in MHB, LHB and VMH from tumor-bearers. However, several of these alterations were similarly observed in brain nuclei from pair-fed controls. Provision of unspecific NOS-antagonists to TB mice increased nNOS, eNOS and iNOS in several brain nuclei (PVN, LHA, VMH), but left tumor-induced anorexia unchanged. c-fos was significantly increased in all brain nuclei in PF mice except for NTS, LHA and PVN compared to controls, while tumor-bearing mice had increased c-fos in LHA and PVN only compared to controls. Our results demonstrate a complex picture of NOS expression in brain areas of relevance for appetite in tumor-bearing hosts, where most changes seemed to be secondary to stress during negative energy balance. By contrast, NOS content in PVN and LHA nuclei remains candidate behind anorexia in tumor disease. However, nitric oxide does not seem to be a primary mediator behind tumor-induced anorexia. NO may rather secondarily support energy intake in conditions with negative energy balance.

Yabuno, T., N. Konishi, et al. (1998). "Drug resistance and apoptosis in ENU-induced rat brain tumors treated with anti-cancer drugs." J Neurooncol **36**(2): 105-12.

To cast light on the mechanisms of drug-resistance, experimental brain tumors were immunohistochemically evaluated for expression of glutathione S-transferase (GST)-alpha, mu, pi, p-glycoprotein and apoptosis-related factors, such as bcl-2 and p53, as well as by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling (TUNEL) method. Rat brain tumors induced by means of prenatal exposure to ethylnitrosourea (ENU) were treated with 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU) and/or vincristine. Tumors more than 2 mm in size were considered to be drug resistant. The expression of GST-mu was strongly positive in ACNU-treated brain tumors, while p-glycoprotein was overexpressed in vincristine-treated brain tumors. Neither p53 nor bcl-2 expression directly correlated with apoptosis identified by TUNEL method, but tumors lacking apoptotic cells always demonstrated the expression of either GST-mu or p-glycoprotein. These results indicate that tumors resistant to chemotherapy might not be susceptible to induction of apoptosis, and therefore that mechanisms of drug resistance are related to programmed cell death in brain tumors.

Zhang, Y., Y. F. Zhang, et al. (2004). "Intravenous RNA interference gene therapy targeting the human epidermal growth factor receptor prolongs survival in intracranial brain cancer." Clin Cancer Res **10**(11): 3667-77.

The human epidermal growth factor receptor (EGFR) plays an oncogenic role in solid cancer, including brain cancer. The present study was designed to prolong survival in mice with intracranial human brain cancer with the weekly i.v. injection of nonviral gene therapy causing RNA interference (RNAi) of EGFR gene expression. EXPERIMENTAL DESIGN: Human U87 gliomas were implanted in the brain of adult scid mice, and weekly i.v. gene therapy was started at day 5 after implantation of 500000 cells. An expression plasmid encoding a short hairpin RNA directed at nucleotides 2529-2557 within the human EGFR mRNA was encapsulated in pegylated immunoliposomes. The pegylated immunoliposome was targeted to brain cancer with 2 receptor-specific monoclonal antibodies (MAb), the murine 83-14 MAb to the human insulin receptor and the rat 8D3 MAb to the mouse transferrin receptor. In cultured glioma cells, the delivery of the RNAi expression plasmid resulted in a 95% suppression of EGFR function, based on measurement of thymidine incorporation or intracellular calcium signaling. Weekly i.v. RNAi gene therapy caused reduced tumor expression of immunoreactive EGFR and an 88% increase in survival time of mice with advanced intracranial brain cancer. CONCLUSIONS: Weekly i.v. nonviral RNAi gene therapy directed against the human EGFR is a new therapeutic approach to silencing oncogenic genes in solid cancers. This is enabled with a nonviral gene transfer technology that delivers liposome-

encapsulated plasmid DNA across cellular barriers with receptor-specific targeting ligands.

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