

# 556 PTEN can suppress NSCLC invasion through PI3K/AKT/NFkB pathway

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**Background:** It is now widely accepted that human carcinogenesis is a multi-step process and phenotypic changes during cancer progression reflect the sequential accumulation of genetic alteration in cells. Thus, in order to understand the process of acquisition of metastatic phenotypes in cancer cells, it is indispensable to identify genes whose alterations accumulate during cancer progression and correlate with metastatic phenotypes of cancer cells. For this reason researchers have been searching for genes that are preferentially altered in invasive & metastatic cancer cells and have activities to regulate their invasive & metastatic potentials. In non small cell lung cancer (NSCLC) PTEN/MMAC1 gene is frequently inactivated. However, it still remains unclear whether this gene is involved in the regulation of metastatic potential in lung cancer cells.

**Material and Methods:** PTEN cDNA isolated from cDNA library and then cloned tetracycline inducible vector. PTENG129R and PTENG129E mutants are established by site direct mutagenesis reaction. Stable transfection conformed by FUGEN HD and NFkB activity detected by using Luciferase gene reporter system, expression levels of PTEN, Akt, Betacatenin and E-Cadherin were detected by western blot.

**Results:** Our data have shown that PTEN expression is very important for normal lung cells and a lot of lung cancer cell lines do not express the PTEN tumour suppressor gene, and there is a correlation between PTEN expression loss and increased AKT activity. We also found that stable wt PTEN expression, which was created stable transfection in no PTEN expressed NSCLC, is suppressed the cell invasion at 72%. For the determination of the mechanisms, we created PTENG129R and PTENG129E mutants vectors which are catalytically inactive and lipidphosphatase death PTENs respectively on tetracycline inducible vector. Constitutive expression of wild type PTEN, but not neither catalytically inactive mutant (PTENG129R) or Lipid phosphatase death mutant (PTENG129E) reduced phosphorylation on Ser-473 of AKT and the activation of NFkB, and the cellular invasion in NSCLC cell line PC14. We also find that only wt PTEN expression can induce beta catenin expression and reduce E-cadherin expression. Moreover we showed that only continuously AKT expression is enough for the induce invasion on NIH3T3 cells.

**Conclusions:** PTEN tumour suppressor gene product can control Akt and NFkB activation. Knowing that NFkB is a transcriptional activator which regulates survival pathways, and that metastasis is a process related with cell survival, one can comprehend the presence of a possible interaction between metastasis and NFkB activation. We conclude that PTEN is sufficient to reduce the invasive potential of NSCLC cell invasion by lipid phosphatase activity and implies that PI3K/AKT/NFkB/Twist pathway is important to invasiveness on NSCLC.

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# 557 Myelin basic protein in malignant brain tumour – source of increased myelin basic protein in cerebrospinal fluid

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**Background:** We reported that the level of myelin basic protein (MBP) in the cerebrospinal fluid (CSF) increased and reflected the effect of treatment in malignant brain tumours (BTs) (Neurosurgery 34, 1994). We investigated the mechanism of the increased level of CSF MBP pathologically using clinical specimens and experimental BTs under the permission of the Ethics Committee of the Nozaki Tokushukai Hospital.

**Material and Methods:** Surgical specimens of glioma, metastatic brain tumour (MBT), brain tissue adjacent to MBTs, other BTs and cyst fluid of various kinds of BTs were examined by radioimmunoassay. The immuno-histochemical localization of MBP in BTs was also examined and compared with those of glial fibrillary acidic protein (GFAP) and 2',3'-cyclic nucleotide, 3'-phosphodiesterase (CNP). Moreover, immuno-histochemically double-stained specimens using mouse monoclonal anti-GFAP and rabbit polyclonal anti-MBP antibodies were subjected to laser microscopy to confirm the sources of MBP in BT tissue. Finally, MBTs and glioblastomas were investigated by electron microscopy.

**Results:** Malignant glioma cyst fluid showed the highest levels of MBP among the BTs examined. However, the quantitative measurements of tissue MBP showed the highest levels in the brain surrounding MBTs. Immuno-histochemical analysis of MBP in glioma showed marked staining in the brain at the border zone where reactive astrocytes were observed. GFAP immunohistochemistry also showed marked staining in the same region, while staining for CNP was weak. In MBTs, areas of normal brain tissue close to tumour showed marked GFAP staining, and MBP and CNP staining were confirmed in areas close to the strongly GFAP-immunolabeled regions. On electron microscopic analysis in the brain tissue adjacent to tumour, degenerated myelin due to massive edema fluid and phagocytosis of

degenerated myelin by macrophage or microglia in MBT, and oligodendrocytes surrounded by many myelin as well as degenerated myelin in glioblastoma were observed.

**Conclusion:** From these findings, we speculated that MBP is produced by oligodendrocytes around tumour cells. On the other hand, MBP is released from injured oligodendrocytes in the process of degeneration by compression of tumour cells or edema fluid. We also speculate that the increased MBP in the cerebrospinal fluid was due to induction of release from microglia and macrophages by phagocytosis of degenerated myelin.

# 558 Epstein Barr virus latent membrane protein 1 in Hodgkin lymphoma

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**Background:** Epstein-Barr virus (EBV) is now known to be firmly associated with Hodgkin lymphoma (HL) and is detected in more than 50% of patients. Studies have shown that EBV-positive Reed-Sternberg cells express the latent membrane proteins (LMPs) which possess oncogenic properties and may lead to the activation of signalling pathways like MAP kinases (MAPK) and phosphatidylinositol-3 kinases (PI3K). Alterations of these signalling pathways in the presence or absence of viral factors, such as LMPs have not been reported in Hodgkin's lymphomas. The working hypothesis of the project is that LMP1 expression in lymphocytes will lead to the activation of the MAPK and PI3K pathways which are associated with the pathogenesis of HL.

**Material and Methods:** Plasmid harbouring LMP1 and empty vector was transfected into Hodgkin lymphoma cell line. A small volume of cells was harvested to check the transfection efficiency by flow cytometer and Western blot analysis to confirm the specificity of the expressed protein. A sample of cell lysate protein was subjected to SDS-PAGE before blotting onto nitrocellulose membrane to detect and quantify the phosphorylated status of major proteins involved in MAPK and PI3K pathways. Immunohistochemistry was performed on HL EBV-positive and HL EBV-negative tissues to show the immunophenotype changes, cytoplasmic and/or nuclear localization of activated MAPK and PI3K pathways.

**Results:** The ERK, JNK, p38 MAPK and Akt pathways were constitutively activated in Hodgkin cell lines and RS cells of Hodgkin tissues. Although p44 and p42 are both expressed at high levels in L428, L591 and KMH2 cells, only p42 was strongly phosphorylated in KMH2-EBV cells. C-terminal activation regions of LMP1 play an important role in activating both JNK and p38 MAPK pathways. We found isoforms JNK2 and JNK3 were highly expressed in all cell lines as compared to JNK1. However, JNK1 and JNK2 were detected in both nucleus and cytoplasm of RS cells of all tissues. Our study showed that phosphorylation of JNK was driven by LMP1. It was reported that JNK appears to have a unique prosurvival role. We reported that from the immunohistochemistry analysis, p38 MAPK was dual-phosphorylated at Thr180/Tyr182 and subsequently activated ATF-2. Akt2 which stimulates cell motility was expressed in all cell lines and tissues compared to Akt1 and Akt3. We believe differential regulation of Akt isoforms must be functionally important in the biology and survival signalling of RS cells. Phosphorylation of Akt at Ser473 was also highly expressed in tissues and KMH2-EBV but not in L428 cells. We found that isoform GSK3 $\alpha$  was instead abundantly expressed and activated in Hodgkin cell lines especially KMH2-EBV and tissues.

**Conclusion:** We suggest that these signalling cascades are involved in the pathogenesis of Hodgkin lymphoma. Collectively, our data demonstrate that EBV LMP1 play an important role in activation of ERK, JNK, p38 MAPK and Akt pathways to maintain its survivability in Hodgkin tumours.

# 559 Galectin-1 expression in Hodgkin-lymphoma cells

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**Background:** Galectin-1 (gal-1) is a carbohydrate-binding protein, a regulator of immune responses and T-cell homeostasis. The mechanisms modulating gal-1 expression are still largely unknown. Gal-1 expression or overexpression was observed in several human tumours. The aim of our study was to examine gal-1 expression in different Hodgkin-lymphoma (HL) samples – both in tumour cells and in the stroma –, and to determine regulator T-cell phenotype in the microenvironment. Furthermore *in vitro* and *in vivo* models were initiated for the investigation of gal-1 expression after treatments.

**Materials and Methods:** The expression of different proteins was examined in tumour cells and in their microenvironment in formalin fixed and paraffin embedded HL samples (from 93 patients) by immunohistochemistry (IHC) (gal-1, CD4, Foxp3). Gal-1 was examined in several lymphoma/leukemia cell lines by immunocytochemistry (ICC). A HL cell line (KMH2) was cultured and treated *in vitro* and xenotransplanted into SCID mice. The effect of several