



PTEN Literature Review

Introduction

The PTEN gene (phosphate and tensin homolog deleted on chromosome 10, also known as MMAC1) is a tumor suppressor located at 10q23⁶¹. PTEN is a key regulator of cell signaling pathways that regulate growth and survival. Specifically, PTEN is a lipid phosphatase that converts PIP3 (phosphatidylinositol 3-4-5 triphosphate) to PIP2 (phosphatidylinositol 3-4 biphosphate). This enzymatic activity is antagonized by PI3K (phosphoinositide 3-kinase) and it is the balance of these two activities which tightly regulates the levels of phosphorylated phosphatidylinositol. When the levels of PIP3 rise, downstream pathway targets are activated leading to cell growth^{62, 63}. In tumors, PTEN is often nonfunctional resulting in the expression of the active form (pAkt) of the proto-oncogene Akt, cell cycle progression and inhibition of apoptosis^{64, 65}. Akt phosphorylation activates mTOR (mammalian target of rapamycin) resulting in 4E-BP1 and p70S6K phosphorylation and an up-regulation of cell cycle proteins⁶⁷.

PTEN aberrations have been associated with human disease including cancer. Functional deficits in PTEN may be due to germline mutations that cause rare autosomal dominant inherited cancer syndromes like Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. Patients with these syndromes have a high risk of developing cancers of the breast, thyroid and endometrium.

More commonly, loss of PTEN function is the result of somatic mutations, deletions or promoter methylation seen frequently in glioblastoma, prostate cancer, melanoma and endometrial cancer. PTEN mutations have also been found, though not as frequently in cancers of the colon, lung, breast, ovary, bladder, kidney and lymphatic system⁶⁶. In general, loss of PTEN has the anticipated effect of increased Akt phosphorylation, however, there are indications that novel regulatory mechanisms may be found in some tumor types as evidenced by the coexistence of high PTEN expression and pAkt in renal cell carcinoma^{125, 126}. The clinical implications of PTEN mutations and loss of protein expression in specific cancers are reviewed below.

Prostate Cancer

In prostate cancer PTEN status has been correlated with disease progression and the onset of androgen independent growth. One of the first studies to demonstrate a correlation between PTEN and disease progression was done in mice and found PTEN to be haplo-insufficient in tumor suppression¹. Since then, several groups have looked at human tumors and have found a correlation between PTEN status and disease progression^{2, 3, 27, 59, 68}. A FISH analysis found a significant correlation between PTEN loss and earlier onset of progression particularly for homozygous deletions ($p= 0.002$)²⁷. The likelihood of progression due to a TMPRSS2: ERG gene fusion was found to increase with simultaneous PTEN loss⁶⁸. These two aberrations frequently occur together and facilitate the transition from HGPIN (high grade prostatic intraepithelial neoplasia) to cancer^{150, 151}.

In androgen dependent metastatic prostate cancer treated with hormonal therapy, high PTEN and androgen receptor expression were associated with increased relapse free survival; 86% at 30 months vs. 17% for low expression¹⁰¹. PTEN loss has also been correlated with androgen independent growth in mouse models⁴⁻⁶ and in human tumors⁷. In a study of hormone refractory prostate cancer, PTEN deletions were common (77% of tumors) and were significantly associated with disease specific mortality¹⁴⁹. Recently PTEN status was found to be associated with response to EGFR inhibitors in androgen independent prostate cancer⁶⁹.

Breast Cancer

Several studies have been published in which PTEN loss was associated with resistance to Herceptin in HER2 positive breast cancer patients and cell lines^{8-10, 144}. PIK3CA mutations and PTEN loss are frequently concordant^{58, 73} and have been found to predict poor prognosis and risk of progression following Herceptin based therapy^{28, 43}. PTEN loss was not associated with resistance to lapatinib in breast cancer patients or in some cell lines¹⁴²⁻¹⁴⁴ but one group found an association with resistance in cell lines and xenografts which was reversed by the addition of a dual PI3K/ mTOR inhibitor¹⁴¹. This finding is consistent with other studies in which PTEN loss has been correlated with sensitivity to mTOR inhibitors. Studies in mice and cell lines have found evidence for sensitivity to mTOR inhibitors^{70, 71, 57, 67} and PI3K inhibitors^{58, 67} along with possible resistance to doxorubicin⁵⁷. However the

association of PTEN loss with mTOR inhibitor sensitivity has not been established in human clinical trials and a recent study suggests a role for the tumor suppressor FBXW7¹³⁵.

In addition to the correlations with drug response, several studies have found PTEN to be associated with prognosis. In a study of node-negative breast cancer treated with CMF (cyclophosphamide, methotrexate, 5-fluorouracil) adjuvant therapy, PTEN expression was associated with survival independently and in combination with low mitotic activity index¹¹. PTEN loss has also been associated with breast cancer that is aggressive and high grade. In a mouse model of HER2 positive breast cancer, loss of PTEN accelerated the induction of breast cancer²⁹ and in humans PTEN loss was associated with high grade carcinoma³⁹. Inactivation of PTEN resulted in the formation of basal like tumors in mice and was significantly associated with basal like cancer in human sporadic and BRCA1 associated hereditary cases⁴⁰.

Glioblastoma

In glioblastoma, PTEN loss has been associated with disease progression as a part of a genetic model requiring mutation in one or more additional genes (PAX6, EGFR, VEGF, IGFBP2)¹²⁻¹⁵. Immuno-resistance is thought to play a role in progression and one study found an increase in the expression of immunosuppressor B7-H1 after loss of PTEN⁴¹. Although PTEN mutations are frequent in primary glioblastoma they are rare in secondary glioblastomas arising from a low grade prior glioma. However, in secondary tumors and astrocytomas frequent PTEN promoter methylation has been observed resulting in increased PI3K signaling⁴⁸.

Several groups have examined the association of PTEN with response to glioblastoma therapies with conflicting results. In patients with recurrent glioblastoma treated with EGFR inhibitors co-expression of EGFRvIII and PTEN were significantly associated with response¹⁶. But in another study of an EGFR inhibitor (Tarceva with carboplatin) in recurrent patients PTEN expression was not associated with progression free survival or overall survival⁷⁴. In cell lines sensitivity to the EGFR inhibitor Iressa was achieved, irrespective of PTEN or EGFRvIII status, by adding Lovastatin which targets HMG-CoA reductase¹³⁷. PTEN loss has also been associated with resistance to radiation and temozolomide in cell lines and a mouse model¹⁷ but no association between PTEN status and radiation response was seen in mouse xenografts¹⁸. PTEN loss was not sufficient to predict response to an mTOR

inhibitor (RAD001) in a mouse xenograft study of glioblastoma⁷⁵ or in patients with recurrent disease and PTEN loss where 50% did not respond to the mTOR inhibitor rapamycin but retained sensitivity ex-vivo⁷⁶. In a study of two glioblastoma cell lines results suggested that PTEN positive glioblastoma may be sensitive to Taxol¹³⁶.

Colorectal Cancer

The first study of PTEN in colorectal cancer (CRC) looked at the frequency of PTEN mutations in a small cohort of patients with microsatellite instability (MSI) and TGFβ receptor II mutations. PTEN mutations were frequent (60%) in this cohort³⁰. In a larger cohort of MSI CRC patients, PTEN promoter hypermethylation (19% of patients) was significantly associated with decreased or loss of PTEN protein expression³⁸. PTEN expression was also found to be negatively associated with young age, female sex, and left sided (distal) tumors with loss associated with local recurrence⁶⁰.

Three recent studies found loss of PTEN protein expression alone or with KRAS mutations to be significantly associated with non-response to Erbitux and or Vectibix in metastatic CRC patients^{42, 100, 146}. Similar associations between PTEN loss and resistance to treatment were seen in additional cohorts of metastatic CRC patients treated with Erbitux combined with irinotecan or other chemotherapy^{45, 134}. Colon cancer cell lines with loss of PTEN expression, PIK3CA mutations, or Ras/ Braf mutations were highly resistant to Erbitux⁴⁴. However, it is unclear whether PTEN status is conserved between primary and metastatic tumors. In a follow up to the first study⁴² to demonstrate an association with Erbitux response, PTEN status was found to be concordant in 29/32 (91%) of primary tumors and paired metastases⁴⁶. Another group reported a lower rate of concordance 30/45 (67%) and found PTEN to be associated with Erbitux response only in metastases⁴⁵.

Non-Small Cell Lung Cancer

In non-small cell lung cancer (NSCLC) mutations in PTEN are rare but PTEN protein loss due to LOH or promoter hypermethylation occurs relatively frequently. PTEN loss has been associated with aggressive disease and reduced survival^{20, 21}. There are conflicting reports regarding whether PTEN loss is an early or late event with some indications of variance by histological subtype²². Additionally, there is some data suggesting that the association of PTEN protein loss with prognosis may be

restricted to later stages^{19, 23}. One group found PTEN transcriptional up-regulation to be associated with worse prognosis in female patients with NSCLC³⁶ but they did not examine protein levels and therefore their results cannot be compared with the other studies.

The role of PTEN in lung cancer tumorigenesis is under investigation. In mice with a bronchiolar epithelial specific PTEN null mutation, PTEN was found to be essential for normal lung development and the prevention of lung carcinogenesis³⁵. A more recent study, again using a mouse model to research the role of PTEN in lung cancer development, found PTEN inactivation alone had no effect upon bronchial epithelial histology. However, PTEN loss accelerated lung tumorigenesis initiated by KRAS mutations resulting in a significant reduction in median survival (8 weeks vs. 24 weeks), increased tumor vasculature and higher histological grade³³.

Several reports suggest that PTEN may play a role in NSCLC response to EGFR inhibitors. Reduced PTEN protein expression has been seen in NSCLC adenocarcinoma cell lines that are resistant to Iressa²⁴ and treatment of resistant cell lines with a demethylation agent and a histone deacetyltransferase inhibitor increased PTEN expression and Iressa sensitivity⁴⁷. In two cohorts of NSCLC patients treated with Iressa PTEN expression was associated with longer overall survival^{31, 37}. Pretreatment of EGFR inhibitor sensitive NSCLC cells with cisplatin reduced PTEN function, activated the PI3K/ AKT pathway in an investigation of the mechanism of resistance to EGFR inhibitors¹⁴⁰. A recent study found that homozygous deletions of PTEN resulted in resistance to Tarceva in EGFR mutant NSCLC cell lines and tumors¹⁴⁷.

Melanoma

Mutations in the PTEN coding region that result in frameshift or stop codons have been identified in primary and metastatic melanoma tumors⁷⁸ but more frequently PTEN loss is due to LOH, homozygous deletion or promoter methylation. In a study of 76 melanoma cell lines PTEN LOH was observed in 55% and homozygous deletion was observed in 10%⁸³; similar findings were reported in another melanoma cell line study⁸⁴.

PTEN loss occurs more frequently in metastatic than primary tumors and within the cells loss of expression is seen more frequently in the nucleus than the cytoplasm⁷⁹. High levels of PTEN promoter methylation were found in tumors and sera from

metastatic melanoma patients and in an in-vitro assay the reductions in protein expression were reversible by treatment with a demethylating agent ⁸².

PTEN expression in mouse melanoma cells halted tumorigenesis demonstrating the critical role of PTEN in melanoma development ⁸⁰ and studies in human tumors found a correlation between PTEN loss and reduced disease free and overall survival ⁸¹.

Endometrial Cancer

Endometrial cancer is common in patients with Cowden's syndrome (a rare autosomal dominant disorder caused by germline mutations in PTEN). Somatic PTEN mutations are common in sporadic endometrial cancer; they may occur alone, with PI3CA ^{93, 96} or FGFR2 (fibroblast growth factor receptor 2) ¹³³ mutations, and are most frequent in tumors exhibiting microsatellite instability (MSI) ⁸⁵. PTEN mutations have also been found in precancerous conditions such as complex atypical hyperplasia suggesting these mutations may occur early in endometrial carcinogenesis ^{86, 88}. In analyses of PTEN mutations, variants were associated with endometrioid histology, early stage disease and other favorable clinical characteristics ^{87, 92} though these associations may be restricted to mutations outside exons 5-7 ⁹⁰ and tumors without MSI ⁹⁵.

PTEN promoter methylation and loss of protein expression are observed fairly frequently in endometrial cancer but there are indications that their association with outcome may differ from PTEN mutations. PTEN promoter methylation has been associated with advanced stage and metastasis ⁸⁹. Loss of PTEN protein expression has been associated with significantly lower (12% vs. 62%) survival rates in patients with advanced endometrial cancer receiving adjuvant chemotherapy ⁹¹ and for patients with early stage disease ⁹⁹. Low PTEN protein expression has been correlated with exon 8 mutations in patients with ≥ 2 mutations and with promoter methylation plus mutation ⁹². In precancerous hyperplasia PTEN loss was more frequent (41% vs. 6%) in cases resistant to progesterone treatment as was phosphorylation of mTOR ⁹⁷. There is some evidence to indicate that PTEN status may affect endometrial cancer sensitivity to Iressa ⁹⁸.

Ovarian Cancer

The investigation of the role of PTEN in ovarian cancer and in normal ovarian function has only recently begun. In a study of ovarian function PTEN was disrupted

in granulosa cells resulting in corpus lutea that persisted significantly longer than in wild type mice, suggesting PTEN impacts the survival/ life span of luteal cells⁵⁶. Another study in mice with PTEN null oocytes found that the entire primordial follicle pool was depleted resulting in premature ovarian failure³⁴. These studies suggest loss of PTEN may result in abnormal ovarian function though the link with carcinogenesis is unknown. PTEN mutations have been identified in ovarian cancer and were found to be more common in low grade cancers of endometrioid histological subtype¹¹¹. In two studies of primary ovarian cancer tumors PTEN protein loss was associated with early stage (I/ II) disease of endometrioid subtype^{55, 113} and longer progression free survival⁵⁵. However, another study of 151 epithelial ovarian cancer patients found a significant association between reduced PTEN expression and shorter disease free survival¹⁴⁵.

The role of PTEN in resistance to ovarian cancer therapies has been investigated primarily in cell lines. Cisplatin sensitive lines had higher PTEN expression which could be reduced by transfection with PTEN shRNA resulting in cisplatin resistance. Similarly, cisplatin resistant cell lines transfected with wild type PTEN experienced an increase in PTEN expression and cisplatin sensitivity^{54, 112, 114}. Data from a recent study of micro RNAs in ovarian cancer found miR-214 induced cisplatin resistance by targeting the 3' UTR of PTEN resulting in Akt activation. The use of an Akt inhibitor or the introduction of PTEN cDNA lacking the 3' UTR restored sensitivity to cisplatin¹¹⁰. Cell lines with high PTEN expression had reduced topoisomerase I activity and 6.6 fold greater sensitivity to irinotecan¹¹⁵.

Hepatocellular Carcinoma

PTEN mutations have been found in hepatocellular carcinoma (HCC) some of which result in loss of function^{102, 49}. PTEN protein loss has been observed in 30-57%^{49, 103, 104} of HCC tumors with normal PTEN expression in surrounding healthy tissue^{49, 108}. Reduced PTEN protein expression has been significantly correlated with high grade¹⁰⁸, advanced stage, p53 overexpression, higher recurrence rate and shorter overall survival^{103, 104, 109}. Recently PTEN has been shown to be inhibited by micro RNA miR-21 which is frequently overexpressed in HCC¹⁰⁶.

The role of PTEN in carcinogenesis was examined in PTEN null mice. This study found high rates of adenoma (100% by 74-78 weeks) and HCC (66%) often preceded by hepatomegaly and steatohepatitis, implying a role for PTEN in liver

function and tumorigenesis ^{105, 107}.

PTEN loss has been linked with resistance to EGFR inhibitors in other cancers and this association has been investigated in HCC. In a study comparing HCC cell lines sensitive to Iressa induced reduction of angiogenesis with resistant lines PTEN concentration was 50% lower in the resistant lines. Transfection of sensitive lines with PTEN shRNA resulted in a reduction of inhibition of angiogenesis by Iressa ²⁵.

Mesothelioma

As in several other cancers, PTEN status has been linked with prognosis in mesothelioma. Tissue microarrays from 206 patients with mesothelioma were examined for PTEN expression by IHC and patients with PTEN expression were found to have significantly longer survival times than those with PTEN loss ³².

Leukemia

PTEN mutations are not frequent in leukemia with the exception of T-cell acute lymphoblastic leukemia (ALL) ¹²¹ but loss or reduction of PTEN protein expression is fairly common. In a cohort of B-cell chronic lymphocytic leukemia (CLL) patients 24% had no PTEN expression and in another 20% of patients expression was reduced. PTEN protein loss was not due to LOH and no mutations were found in the PTEN gene ¹¹⁶. An alternate mechanism of PTEN inactivation is phosphorylation of its c-terminus regulatory domain which may be caused by casein kinase 2 over expression as is seen in T-ALL¹³⁸, and is frequent (74%) and associated with reduced overall survival in acute myeloid leukemia (AML)¹¹⁷.

There is some evidence that PTEN may play a role in the development of leukemia. PTEN loss in hematopoietic cells resulted in myeloproliferative disorder and leukemia in cellular assays and in mice. PTEN deletion also resulted in proliferation of abnormal hematopoietic stem cells which have an impaired ability to sustain hematopoietic reconstitution ^{119, 120}.

PTEN status has also been associated with response to treatment. In a study of patients with Ph+ ALL who were resistant to Gleevec, PTEN expression was reduced due to promoter hypermethylation. Treatment of Ph+ ALL cells with 5 – aza-2' deoxycytidine resulted in increased PTEN expression and apoptosis in response to Gleevec treatment ⁵⁰. Loss of PTEN expression has also been linked to resistance to

doxorubicin ¹¹⁸ and gamma secretase inhibitors ¹²¹ in ALL cell lines.

Renal Cell Carcinoma

There are conflicting reports regarding the frequency of PTEN protein loss in renal cell carcinoma (RCC) and the resulting clinical implications. In a cohort of 80 clear cell RCC patients LOH at or near PTEN was found in 37% and was associated with increased rates of death from RCC (85.7% vs. 45.3%) ¹²². Similarly, PTEN expression was associated with disease specific survival ($p=0.0001$) in a cohort of metastatic RCC patients ¹²³ while in other studies the association with survival was not as strong ($p=0.02$) ¹²⁴ or was absent ¹²⁷. Recently, PTEN expression was found to be significantly elevated in RCC ($p=0.0001$) when compared with adjacent benign tissue. However, PTEN overexpression did not inhibit phosphorylation of Akt ¹²⁵. This coincidence of high levels of PTEN and pAkt confirms previous results ¹²⁶ and suggests that novel mechanisms may regulate these two proteins in RCC.

Researchers have started investigating the role of PTEN in response to RCC therapies. In a cohort of RCC patients treated with neoadjuvant Avastin and Tarceva a distinct pattern of protein expression was found in the tumors that shrank $\geq 10\%$. Of the top eight proteins with altered expression, high PTEN expression had the most significant correlation with tumor shrinkage ($p= 1.3 \times 10^{-7}$) ⁵¹.