PTEN MUTATION IS PRESENT IN AN EXTRASKELETAL MYXOID CHONDROSARCOMA

Introduction

PTEN is a recently identified tumor suppressor gene. The PTEN gene is mutated or deleted in malignant tumors such as brain, breast, prostate, and endometrial carcinomas and melanoma, and is mutated in high grade but not low grade gliomas. PTEN is located on chromosome 10q23. Previous studies have shown loss of heterozygosity of 10q in chondrosarcoma, suggesting the presence of a tumor suppressor gene in this region. In an ongoing search for the molecular genetic underpinnings of chondrosarcoma, we surveyed a series of chondrosarcoma, including an extraskeletal myxoid chondrosarcoma (EMC), for PTEN mutations. EMC is one type of chondrosarcoma which arises in the soft tissues and although has a benign histologic appearance, has a tendency to develop late metastases. EMC have one of two translocations: t(9;22)(q22;q12) or t(9;17)(q22;q11). Both translocations result in a fusion protein with an RNA-binding N-terminal domain and an orphan nuclear receptor in the C-terminal domain. The hypothesis is that PTEN mutations are present in some high grade chondrosarcoma.

Methods

RNA isolation. Total RNA was extracted from 13 cartilage lesions (1 chondroblastoma; 3 grade I, 6 grade II, and 2 grade III conventional chondrosarcoma; and 1 EMC) and 3 chondrosarcoma cell lines. Samples of human tumors were acquired from surgical specimens and flash frozen in liquid nitrogen. RNA was extracted by homogenizing the tissue in ice-cold 4 M guanidinium isothiocyanate buffer. The extract was then centrifuged at 10000 x g for 30 minutes and the supernatant was layered on a cesium trifluoride cushion and centrifuged at 35,000 at 200C for 18 hours. The RNA pellet was washed with ethanol, dissolved in 0.1% diethylpyrocarbonate water, and quantified with UV spectrophotometry. Ultraspec™ RNA purification reagent (Biotecx, Houston, TX) was used to isolate RNA from cells after one week in monolayer culture.

RT-PCR Five ug of total RNA were reversed-transcribed into cDNA using Superscript II reverse transcriptase and random hexamers according to the manufacturer’s instructions. The entire PTEN cDNA was amplified using the expand high-fidelity PCR system for 35 cycles and 30 s at 94C, 20 s at 55C and 2 min at 70C. The primers used were as reported3. There are 2 subtypes of expand high-fidelity PCR system for 35 cycles and 30 s at 94C, 20 s at 55C and 30 s at 72C. Previous studies have shown loss of heterozygosity of 10q in chondrosarcoma3, suggesting the presence of a tumor suppressor gene in this region. In an ongoing search for the molecular genetic underpinnings of chondrosarcoma, we surveyed a series of chondrosarcoma, including an extraskeletal myxoid chondrosarcoma (EMC), for PTEN mutations. EMC is one type of chondrosarcoma which arises in the soft tissues and although has a benign histologic appearance, has a tendency to develop late metastases3. EMC have one of two translocations: t(9;22)(q22;q12) or t(9;17)(q22;q11). Both translocations result in a fusion protein with an RNA-binding N-terminal domain and an orphan nuclear receptor in the C-terminal domain. The hypothesis is that PTEN mutations are present in some high grade chondrosarcoma.

Results

PTEN was present and of normal size in all of the chondrosarcoma specimens and cell lines except for the EMC. In the EMC, normal sized and truncated PTEN was present (Figure 1).

Figure 1. Results of a protein truncation test are shown for five chondrosarcoma. Lane four, which is from EMC, has a truncated protein indicated by the arrow.

DNA sequencing analysis of PTEN identified two mutations: an insertion of A at position 406, and a T to G substitution at position 522. The frame shift from the insertion results in a premature stop codon at position 534 with a predicted truncated PTEN of 177 amino acids. The result from PTT matched the predicted size of the truncated PTEN.

To confirm the diagnosis of EMC, primers specific for the type 1, type 2 EWS/CHN fusions and the TAF2N/TEC fusions were used in the RT-PCR analysis. The results showed that EMC had the type 2 EWS/CHN fusion.

Discussion

A PTEN mutation has been identified in ESM, but not conventional chondrosarcoma. EMC tends to be a slow growing tumor but has a propensity for late recurrence and metastasis2. The fusion of EWS to CHN is well documented and is present in about 75% of EMC. The remaining 25% can be accounted for by the fusion of the EWS-related gene TAF2N to CHN-related gene TEC. We have identified an EMC with a type 2 EWS/CHN fusion as well as a truncated PTEN protein.

Consistent with the multiple gene mutation paradigm in carcinogenesis, this EMC has mutations in the tumor suppressor gene PTEN in addition to the typical chromosomal translocation. PTEN is a tyrosine phosphatase which negatively controls the phosphoinositide 3-kinase signaling pathway for cell growth and survival and its mutations have been implicated in a number of advanced malignant tumors. The mutation rate approaches that of p53. This EMC was from a patient who presented with pulmonary metastases. We speculate that the loss of functional PTEN in this EMC may be associated with the metastatic phenotype. It will be of interest to study a larger series of chondrosarcoma and EMC to determine the generalizability and specificity of this finding.

Reference