METHYLTHIOADENOSINE PHOSPHORYLASE (MTAP) GENE DELETION IN OSTEOSARCOMA

*Garcia-Castellano, J; *Villanueva, A; +*Healey, J; *Sowers, R; *Huvos, A; *Bertino, J; *Meyers, P; *Gorlick, R
+*Memorial Sloan-Kettering Cancer Center, New York, NY. 1275 York Avenue, New York, NY 10021, (212) 639-7610, Fax: (212) 794-4015, healeyj@mskcc.org

Introduction. Methylthioadenosine phosphorylase (MTAP) is an enzyme essential in the salvage of approximately 90% of the cellular adenine and methionine (Figure 1). MTAP has been implicated in the development, progression and treatment response of multiple neoplasias, including leukemias (2), carcinomas (3) and myxoid chondrosarcoma (4).

The MTAP gene is located in the 9p21 chromosomal region. Loss of MTAP may be associated with the codelletion of tumor suppressor genes p15, p16/ p19ARF. Disruption of this purine salvage pathway by MTAP loss with p15, p16/ p19ARF alterations may contribute to the poor prognosis of osteosarcoma patients.

This study is the first to investigate the prevalence of genetic and functional alterations of MTAP gene in osteosarcoma cell lines and a large clinical series of osteosarcomas.

Material and methods. Three osteosarcoma cell lines (HOS, SaOS-2, U2OS) and 96 high-grade osteosarcoma patient samples were analyzed. Deletions of exons 2 to 7 were studied by PCR and mutational analysis by SSCP in genomic DNA. Quantification of message was performed using a fluorescent RT-PCR in the cell lines, and by semiquantitative RT-PCR in the patient samples. β-actin was used a a positive control. Finally, protein expression was measured by Western blot and immunohistochemistry (primary antibody was a kind gift of Dr. D. Carson).

Results. PCR / agarose gel showed no expression of the MTAP gene in at least in one exon in 38 of 96 cases of osteosarcoma patient samples (39%) and in the HOS cell line (Figure 2).

Mutational study did not show point mutations in any of the samples. There was absence of detectable mRNA in all cases in which a deletion was observed in an MTAP exon (Figure 3).

According to the mRNA results, Western-blot showed no protein expression in the patient samples and in the cell lines in which an exon deletion was observed (Figure 4).

Conclusions. This is the first systematic evaluation of MTAP gene alterations in osteosarcoma samples. We conclude:

1. Deletion of MTAP gene occurs in HOS but not in the SaOS and U2OS osteosarcoma cell lines.
2. MTAP gene is commonly deleted in osteosarcoma patient samples (39%, n=96).
3. When one exon of MTAP is deleted no expression of mRNA or protein are observed.

Further studies are necessary to study how MTAP gene status affects on chemotherapy response; and to correlate these biological parameters with patient outcome.

References.

Funding: New York Marathon Limb Preservation Fund