RESEARCH COMMUNICATION

Methylthioadenosine Phosphorylase Expression in Cutaneous Squamous Cell Carcinoma

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Abstract

The methylthioadenosine phosphorylase (MTAP) gene is a tumour suppressor gene, located on chromosome 9p21, 100 kb telomeric of the p15 and p16 genes, which are often deleted in tumor cells. The role of MTAP protein expression in the genesis of cutaneous squamous cell carcinoma (SCC) is currently not known. In a previous study we have shown the frequent occurrence of allelic imbalance / loss of heterozygosity (AI/LOH) in cutaneous SCCs using AI/LOH markers flanking the p15, p16, and MTAP genes and demonstrated reduction in p15 and p16 protein expression in comparison to normal human skin. The present study is a continuation to our previous studies, aimed at determining possible roles played by MTAP protein expression in the genesis of cutaneous SCC. The expression of MTAP protein was detected using immunohistochemical approach in 109 micro array cutaneous SCC and 20 normal human skin tissue samples. The expression of MTAP was not significantly different in the cutaneous SCC cases as compared with normal human skin. This may indicate that MTAP protein expression does not contribute to the genesis of cutaneous SCC.

Key Words: Cutaneous SSC - MTAP - immunohistochemistry

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Introduction

There is a worldwide increase in the incidence of all types of skin cancer, including both malignant melanoma and non melanoma cancers of skin (Leyden and Marks, 2002). Each year around 2,750,000 cases of nonmelanoma and 92,000 cases of melanoma skin cancer manifest worldwide (Annstron and Kricker, 1995). In the United States of America (USA), skin cancer is the most common neoplasm in Caucasians with a lifetime risk nearly equal to that of all other cancers combined (Scotto et al., 1983).

The methylthioadenosine phosphorylase gene (MTAP) is located on chromosome 9p21, 100 kb telomeric of the genes encoding the cyclin-dependent kinase inhibitors p15 and p16, which are often deleted in tumor cells (Mori et al., 1994; Okuda et al., 1995; Nobori et al., 1996). Methylthioadenosine phosphorylase MTAP is an important salvage enzyme for both adenine and methionine. Specifically MTA generated during the synthesis of polyamines is rapidly cleaved by the ubiquitous enzyme MTAP into adenine and 5-methylthioribose-1-phosphate (Pegg and Williams-Ashman, 1969). Adenine is efficiently salvaged to form AMP by adenine phosphoribosyl transferase, and 5-methylthioribose-1-phosphate is converted to methionine by a complex set of oxidations via the intermediate 2-keto-4-methylthiobutyrate (Backlund and Smith, 1981).

MTAP is abundant in all normal cells including erythrocytes (Kamatani et al., 1981; Sahota et al., 1983) and bone marrow stem cells (Yu et al., 1997) but is deficient in several tumor cell lines (Toohey, 1978; Kamatani et al., 1981) and in primary tumors including glioma (Nobori et al., 1996), non-small cell lung cancer (Nobori et al., 1993), acute non-lymphoid leukemia and melanoma (Fitchen et al., 1986).

It has been shown that MTAP serves as a tumour suppressor gene (Traweek et al., 1988; Subhi et al., 2004; Gray et al., 2006). Homozygous or hemizygous deletions of the MTAP gene has been detected in 50% of primary, conventional, grade II chondrosarcomas (Chow et al., 2006). Early studies showed that 30% of human leukemia cell lines revealed loss of MTAP activity (Christopher et al., 2002). A study of non-small cell lung cancer demonstrated that homozygous deletion of MTAP occurred in 38% of samples examined (20). Another study showed that 43 to 75% of the nuclei of primary pancreatic tumor cells had lost at least one copy of MTAP locus (Schmid, 1998). Additionally MTAP promoter hypermethylation has also been associated with reduced MTAP expression in human melanoma cell lines (Behrmann et al., 2003). The frequency of MTAP loss in human cancers has been reported to be 30% in T-cell lymphoma, 38% in both osteosarcoma and non-small cell lung cancer, 14% in both adult T-cell leukemia (ATL) and

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endometrial cancer, 67% in pleural mesothelioma, and 63% in pancreatic cancer cell lines (Chow et al., 2006). Loss of the MTAP gene rivals the 50% frequency of mutations reported for TP53, which is often cited as the most common mutation in human tumors (Hollstein et al., 1991; Levine et al., 1991). These results suggested that MTAP possesses tumor suppressor activity (Gray et al., 2006).

Cutaneous squamous cell carcinomas (SCC) account for about 20% of non-melanoma skin cancers (with basal cell carcinomas accounting for about 80%) but is clinically more significant because of its ability to metastasize (Johnson et al., 1992). Previously we have investigated the presence of allelic imbalance/ loss of heterozygosity (AI/LOH) in cutaneous SCC using microsatelitte marker flanking a number of tumor suppressor genes such as p15, p16, p19 and MTAP genes (Gray et al., 2006). In the above mentioned studies, we have demonstrated that the p15 and p16 proteins expression were reduced in cutaneous SCC in comparison to normal human skin and we concluded that p15 and p16 proteins expression may contribute to the genesis of cutaneous SCC.

To date there is no information on the role played by MTAP protein in the genesis of cutaneous SCC. The present study aimed to investigate the expression of MTAP protein in cutaneous SCC and in normal human skin using immunohistochemical approach. This work is novel as to date; no work has been carried out to elucidate the role played by MTAP protein expression in the development of cutaneous SCC.

Materials and Methods

Tissue Microarray:

Cutaneous SCC and normal human skin tissue microarray slides were purchased from Biomax, (USA) and AccuMax, (Korea). One hundred and nine cutaneous SCC cores and 20 normal human skin tissue cores were analyzed for the expression of MTAP protein. The age and gender and the tumour grade/clinical-pathological details of each patients have been described in our previous report (Moad et al., 2008). Colon carcinoma tissue samples were used as a positive control for MTAP protein expression.

Immunohistochemical detection of MTAP protein expression in cutaneous SCC tissue

Microarrays and normal human skin tissue: For the immunohistochemical detection of the MTAP, the primary antibody used was Mouse anti-MTAP (Abnova, Taiwan). Briefly, before deparaffinization, the tissue microarray slides and control tissue sections were heated at 60ºC for 30 minutes in horizontal position before proceeding to the staining steps. The deparaffinization steps were carried out in xylene. The deparaffinization step was followed by rehydration through graded alcohol to water. Endogenous peroxidase activity was blocked by incubating the sections in two changes of 3% hydrogen peroxide (H2O2) in PBS (pH 7.4) at room temperature. Antigen retrieval, was carried out by using Target Retrieval Solution (Tris-EDTA PH 9.0);[Dako,USA]. The slides were then immersed in Target Retrieval Solution (TRS) and then microwaved for 20 minutes at 600 watt. The slides in TRS were then left to cool using running tap water.

The ChemMate TM DAKO EnVision TM Detection Kit, USA, was used as described in the manufacturer's instructions. Briefly, the slides were incubated with 200 µl of MTAP antibody at a dilution of 1/50 for 90 minutes at room temperature. Then the slides were incubated with secondary antibody (Rabbit anti-Mouse - ChemMate TM DAKO EnVision, Denmark) for 30 minutes at room temperature then rinsed with TBS. Immunostaining was visualized using diaminobenzidine (DAB) [Dako, USA]. Positive staining to MTAP was recognized under light microscope as a brown color stain in the cytoplasm.

Assessment of MTAP Immunostaining

For each array whole slide cores (1.5 mm diameter each core) were assessed either as positive or negative for MTAP immunostain. The results were graded as weak and strong based on the following criteria: Negative: <1% of cells showing cytoplasmic immunopositivity. Positive: Weak, 10% of cells showing cytoplasmic immunopositivity; Strong , > 90% of cells showing cytoplasmic immunopositivity. Assessment of the staining pattern of MTAP protein expression for all the cores was performed by a single independent pathologist, under x40 magnification.

Statistical analysis:

After immunohistochemical analysis of all cutaneous SCC and normal human skin, data were recorded and analyzed statistically. Results are expressed as percentage. Comparison between groups was made using the Fisher's Exact test. A P-value of < 0.05 was considered statistically significant. All calculations were performed using the SPSS-10 for Windows statistical computer package (SSPS, Chicago, IL).

Results

Expression of MTAP was successfully detected using immunohistochemical approach in 129 microarray tissue samples comprising of 109 squamous cell carcinoma tissue samples and 20 normal human skin tissue samples.

Cytoplasmic methylthioadenosine phosphorylase protein expression was detected by immunohistochemistry in a large series of normal skin and cutaneous squamous cell carcinoma. Seventeen samples (85%) from normal skin were positive for MTAP protein (Figure 1) and three

Table 1. Methylthioadenosine Phosphorylase Expression among 129 SCCs and 20 Normal Human Skin tissue Microarrays

	MTAP Immunoreactivity		
	Negative	Weak positive	Strong positive
Normal Skin	3 (15.0%)	15 (75.0%)	2 (10.0%)
SCCs	25 (22.9%)	38 (34.9%)	46 (42.2%)

Fisher's Exact P-value= 0.002

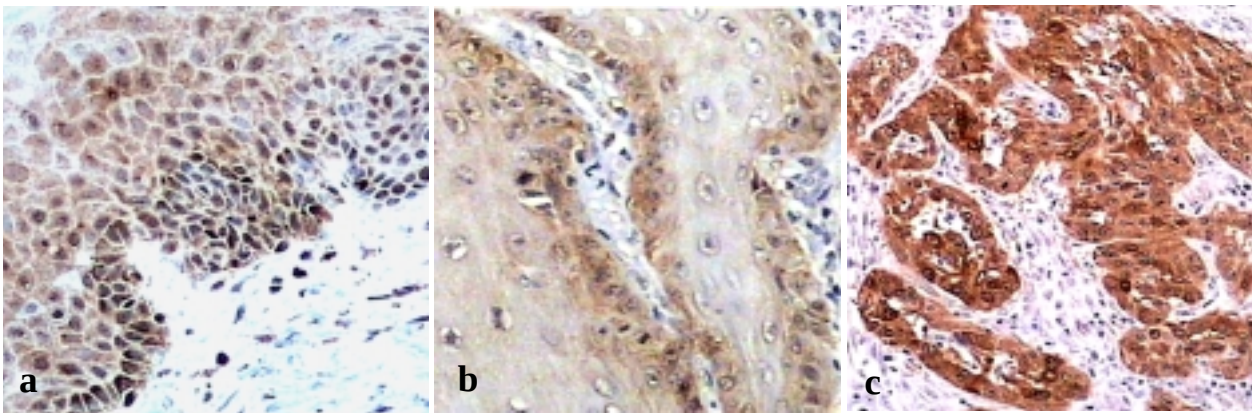

Figure 1. Immunostaining for MTAP Protein. a) Normal Human Skin Demonstrating Positive Cytoplasmatic Staining b) Weak immunostaining in an SCC, c) Strong staining in an SCC, including the nuclei

samples (15%) of normal skin were found to be negative. In regard to cutaneous squamous cell carcinoma, 25 samples (22.9%) were negative, while 84 samples (77.1%) were positive (Figure 1b/c). There was no significant difference of MTAP protein expression between cutaneous SCC and normal skin (P-value= 0.562).

The results for positive normal human skin samples showed only cytoplasmic immunopositivity, but among the squamous cell carcinoma apart from cytoplasmic one case showed nuclear immunopositivity while 16 cases showed a combined pattern.

Intensity of the MTAP protein expression in both squamous cell carcinoma and normal skin samples is shown in Table 1. A significantly greater proportion of the SCCs demonstrated strong staining. There was no significant relation to patient age or sex.

Discussion

The present study showed almost similar results for the expression of MTAP protein in normal human skin and cutaneous squamous cell carcinomas and microarrays of normal tissue samples. In addition, there was no significant relationship between clinicopathologic variables of the patients (age, sex and tumour grade) although more intense expression, also in nuclei, was evident in some of the tumours.

In the present study, MTAP protein expression was detected in almost all (85%) normal human skin samples of the total tissue micro array samples and only (15%) showing negative staining thus supporting the theory of MTAP as an ubiquitous house keeping gene expressed by most normal cells (Subhi et al., 2004). However, expression tended to be higher in cutaneous SCC samples.

Our results for SCCs showed some similarity with the study of Garcia-Castellano et al (2002). They analyzed forty osteosarcoma samples and found immunopositivity for MTAP in 25 cases with strong cytoplasmic immunopositivity in 22 out of 25 positive samples (88%) and weak immunopositivity in 3 out of 25 positive samples (12%). Fifteen samples were immunonegative. Another study by Behrmann and co-workers used immunohistochemical staining on 38 tissue samples of malignant melanomas, metastatic melanoma and benign nevi and revealed a declining pattern of MTAP protein staining from benign nevi to metastatic melanomas and thus demonstrated a significant inverse association between MTAP protein expression and progression of melanocytic tumors (Behrmann et al., 2003).

Tissue microarray (TMA) technology allows a massive acceleration of studies correlating molecular *in situ* findings with clinico-pathological information. Multiple studies have demonstrated that findings obtained on TMAs are highly representative of their donor tissues, despite the small size of the individual specimens (diameter 0.6 mm) (Bubendorf et al., 2001).

Homozygous or hemizygous deletions of the MTAP gene have been detected in 50% of primary, conventional, grade II chondrosarcomas (Chow et al., 2006). These results suggested that MTAP possesses tumor suppressor activity. Early studies showed 30% of human leukemia cell lines revealing the loss of MTAP activity (Christopher et al., 2002). A study of non-small cell lung cancer demonstrated that homozygous deletion of MTAP occurred in 38% of samples examined (Schmid et al., 1998). Another study showed that 43 to 75% of the primary pancreatic tumor cells had lost at least one copy of MTAP locus (Subhi et al.,2004). Previously we have shown a high frequency of allelic imbalance/ loss of heterozygosity (AI/LOH) in cutaneous SCC using microsatelitte marker located located/flanking a number of tumour suppressor genes such as p15, p16 and MTAP (Gray et al., 2006). This suggests that inactivation of the above mentioned genes may be playing a role in cutaneous SCC development. Also, we have shown p15 and p16 protein expression to be reduced in comparison to normal human skin and concluded some contribution to the genesis of cutaneous SCC.

However, from the present results we must conclude that expression of MTAP protein in normal human skin and cutaneous squamous cell carcinomas microarrays tissue samples is found at similar incidence, even if intensity may be greater in some tumours. In addition, there were no significant relationship between clinicopathologic variables of the patients (age, sex and tumour grade) and MTAP protein expression. This may indicate that MTAP does not contribute to the genesis of cutaneous SCC.

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