MDM-2 protein expression in nasopharyngeal carcinomas. Comparative study with p53 protein expression

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Abstract

Aims—To investigate the immuno-histochemical expression of MDM-2 protein in comparison with that of p53 protein in nasopharyngeal carcinomas.

Methods—Formalin fixed, paraffin wax embedded tissue from 59 cases of nasopharyngeal carcinoma was stained by immunohistochemistry for MDM-2 and p53 proteins.

Results—The tumours were divided histologically into seven cases of keratinising nasopharyngeal carcinoma (KNPC), 14 cases of non-keratinising nasopharyngeal carcinoma (NKNPC), and 38 cases of undifferentiated nasopharyngeal carcinoma (UNPC). MDM-2 nuclear expression was observed in 0/7 KNPC, 1/14 NKNPC, and 11/38 UNPC. p53 nuclear expression was observed in 1/7 KNPC, 2/14 NKNPC, and 15/38 UNPC. Parallel MDM-2 and p53 expression was found in 12 cases (11 UNPC and one NKNPC). Discordant MDM-2/p53+ expression was found in six cases (four UNPC, one NKNPC, and one KNPC), and absence of expression of both proteins in the remaining 41 cases.

Conclusions—Expression of MDM-2 and p53 proteins may be associated with the level of tumour cell differentiation in nasopharyngeal carcinoma. Simultaneous expression of MDM-2/p53 in a proportion of UNPC suggests that MDM-2 protein may be responsible for stabilisation of p53 protein in these cases, in view of the previous demonstration of the p53 gene in germ line configuration. This could be important in the pathogenesis of these cases, since MDM-2 may deregulate the p53 dependent growth suppressive pathway. Discordant MDM-2/p53+ expression in a few cases of nasopharyngeal carcinoma may reflect stabilisation of p53 protein by other proteins, or p53 mutations unable to activate MDM-2.

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Alterations of the p53 tumour suppressor gene are the most common genetic abnormalities found in human cancer.¹ p53 mutations associated with overexpression of the gene have been described in a wide variety of human malignant tumours.²⁻⁸ p53 gene mutations

generally lead to stabilisation of the protein which can then be detected by immuno-histochemistry. Stabilisation may, however, also be achieved through binding to other cellular or viral proteins.¹⁻⁹ Indeed, p53 inactivation independent of mutation has been reported in relation to associations with viral oncoproteins such as the SV40 large T antigen, HPV E6 in cervical carcinomas,^{10,11} EBNA-5 protein of EBV,¹² or cellular proteins such as murine double-minute 2 (MDM-2) oncoprotein in some sarcomas.¹³

The MDM-2 oncoprotein was identified in the spontaneously transformed Balb/c 3T3 cell line¹⁴ and when amplified or overexpressed has been shown to increase the tumorigenic potential of cells.¹⁵ The human MDM-2 gene was recently cloned and located on chromosome 12q13-14.¹³ It has been found to be deregulated in human sarcomas, gliomas, breast carcinomas, and leukaemias.^{13,16-19} The mechanism of this deregulation in sarcomas has been shown to be gene amplification,¹³ while in leukaemias significantly high levels of MDM-2 mRNA expression were detected in the absence of MDM-2 gene amplification.¹⁹ It has been suggested that other mechanisms such as alterations of *cis* or *trans* acting factors that regulate the MDM-2 expression may account for the MDM-2 overexpression in such leukaemias.¹⁹ The MDM-2 gene encodes for a nuclear protein which contains two potential DNA metal binding motifs (zinc fingers), and a acid *trans* activator domain, suggesting that it may be a DNA binding protein.¹⁵ MDM-2 protein can bind both wild and mutant p53,^{20,21} and MDM-2 overexpression can inhibit p53 transcriptional activity,²² by concealing the p53 acidic activation domain from the cellular transcription machinery.²³ This may be one of the mechanisms of the transforming potential of the MDM-2 gene which could lead to malignant transformation.

In this study we have used immuno-histochemistry to investigate the expression of MDM-2 protein in comparison to that of p53 protein in a series of 59 nasopharyngeal carcinomas. In a previous study,²⁴ we found that p53 protein expression was more often detected in undifferentiated nasopharyngeal carcinoma, most of which were reported to display germ line configuration of p53 gene.^{25,26} Thus we addressed the question of whether there is immunohistochemical evidence that MDM-2 protein is one of the factors responsible for the

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Table 1 Results of immunohistochemistry

	Number of cases	MDM-2+	p53+
WHO1	7	0	1
WHO2	14	1	2
WHO3	38	11	15

WHO 1, 2, 3 = World Health organisation tumour grade.

stabilisation of p53 protein in undifferentiated nasopharyngeal carcinoma.

Methods

PATIENTS AND TISSUES

In our previous study²⁴ we investigated 44 cases of carcinoma of the nasopharynx retrieved from the 1978–88 files of the pathology department, Evagelismos Hospital, Athens, and from the 1990–1994 files of the department of pathology, University Hospital of Heraklion, Crete. From these 44 cases, only 27 were used for the present study because no more material was available in the remaining cases. In addition, 32 cases were obtained from the department of pathology, Tumour Hospital, Cancer Institute, Sun Yat-Sen, University of Medical Sciences, Guangzhou, People's Republic of China (Dr Dai Yiran). Diagnosis and histological classifications were made on formalin fixed and paraffin embedded sections, stained by haematoxylin–eosin, according to the WHO classification.²⁷

Table 2 Comparison between p53 and MDM-2 expression

		MDM-2 positive	MDM-2 negative	Total
WHO1	p53 positive	0	1	1
	p53 negative	0	6	6
WHO2	p53 positive	1	1	2
	p53 negative	0	12	12
WHO3	p53 positive	11	4	15
	p53 negative	0	23	23

WHO 1, 2, 3 = World Health Organisation tumour grade.

Figure 1 Nuclear staining for p53 protein in a case of undifferentiated nasopharyngeal carcinoma.

IMMUNOHISTOCHEMISTRY

Immunostaining was performed using the alkaline phosphatase–anti-alkaline phosphatase (AAPAP) method as previously described²⁸ for the presence of MDM-2 and p53 proteins. The anti-MDM-2 Mo Ab 1 and the anti-p53 Mo Ab 1801 antibodies (both from Oncogene Sciences) were used at dilutions of 1:100 overnight and 1:100 for three hours, respectively. The bridging rabbit antimouse (Z 259) and AAPAP complexes (D 314) were obtained from DAKO. A microwave heating step in a solution of sodium citrate was performed before incubation with anti-MDM-2 and p53 antibodies. Xylene dewaxed and alcohol rehydrated paraffin wax sections were placed in coplin jars filled with a 0.01M tri-sodium citrate solution, and heated three times in a conventional microwave oven for five minutes at 700 W. After microwave processing, slides were allowed to cool at room temperature for 15 minutes. They were then washed in Tris buffered saline (TBS) pH 7.4 and incubated with the specific antibody. Positive control slides were included in all tests and consisted of paraffin wax sections from Hodgkin's disease known to be positive for p53 and MDM-2 proteins. Negative control slides were prepared by omitting the primary antibody. In all cases the quality of antigenic preservation was tested by vimentin staining.

Results

HISTOLOGY

Nasopharyngeal carcinomas were classified as keratinising squamous cell carcinoma (WHO 1) in seven cases, non-keratinising squamous cell carcinoma (WHO 2) in 14 cases, and undifferentiated carcinomas (WHO 3) in the remaining 38 cases.

IMMUNOHISTOCHEMISTRY

The results are summarised in tables 1 and 2.

p53

p53 nuclear staining was found in 18/59 samples (fig 1). In correlating p53 expression with histological type, positive staining was observed in 1/7 keratinising squamous cell carcinomas, 2/14 non-keratinising squamous cell carcinomas, and 15/38 undifferentiated carcinomas (table 1). p53 positive cells represented 5–20% of tumour cells. In only three cases were more than 20% of tumour cells p53 positive. No immunoreactivity was found in normal nasopharyngeal epithelia, stromal cells, or lymphoid cells.

MDM-2

MDM-2 nuclear staining was found in 12 nasopharyngeal carcinoma cases (11 undifferentiated and one non-keratinising) (fig 2). MDM-2 positive cells represented no more than 5% of the tumour cells and were in all cases less numerous than p53 positive cells. No immunoreactivity was found in normal nasopharyngeal epithelia, stromal cells, or lymphoid cells.

Figure 2 Nuclear staining for MDM-2 protein in a case of undifferentiated nasopharyngeal carcinoma.

Relation between MDM-2 and p53

p53 positive cases accounted for all 12 MDM-2 positive cases and six out of 47 MDM-2 negative cases. The coexpression of p53 and MDM-2 was statistically significant with a χ^2 value of 30,26 ($p < 0.001$) (table 2).

Discussion

The murine MDM-2 gene has been identified as an oncogene in the 3T3 DM cell line, a highly tumorigenic variant of NIH-3T3 cells, on account of its amplification.¹⁵ Human MDM-2 gene has been found to be deregulated in a variety of human malignant tumours.¹⁶⁻¹⁹ Recent evidence suggests that the relation between MDM-2 and p53 is an important growth control mechanism. Indeed, the MDM-2 gene can be transactivated by wild type p53,²⁹ and overexpression of p53 can induce MDM-2 mRNA and protein expression.^{21,22} Moreover, MDM-2 protein can bind to both mutant and wild-type p53 and thus inhibit p53 mediated transactivation and growth suppression.^{22,30} It has been presumed that inactivation of p53 by MDM-2 overexpression may be one of the mechanisms accounting for the transforming potential of the MDM-2 gene which could lead to malignancy.³¹

Prompted by these data we have investigated MDM-2 and p53 protein expression in nasopharyngeal carcinoma. We found that MDM-2 and p53 protein expression were more frequently associated with undifferentiated nasopharyngeal carcinoma. Expression of p53 and MDM-2 protein was rarely found in other histotypes of nasopharyngeal carcinoma. These results indicate that p53 and MDM-2 protein expression may be associated with the level of tumour cell differentiation in nasopharyngeal carcinoma. Two patterns of MDM-2/p53 protein expression were found in nasopharyngeal carcinoma.

(1) There was simultaneous MDM-2/p53 protein expression in 12 cases (11 undifferentiated nasopharyngeal carcinoma and

one non-keratinising nasopharyngeal carcinoma). Since the p53 gene is in germ line configuration in most specimens of undifferentiated nasopharyngeal carcinoma^{25,26} the immunohistochemical detection of p53 protein may reflect stabilisation of the protein resulting from binding to other proteins. In this regard, MDM-2 protein expression could be one of the factors leading to p53 protein stabilisation in undifferentiated nasopharyngeal carcinoma. This could be of interest with regard to the pathogenesis of the MDM-2+/p53+ cases. As MDM-2 can inhibit p53 mediated transactivation,^{22,23,30} it is possible that MDM-2 expression may be involved in the pathogenesis of some undifferentiated nasopharyngeal carcinomas by deregulating the p53 dependent growth suppressive pathway. The expression of MDM-2 protein in a substantial proportion of undifferentiated carcinomas but not in normal nasopharyngeal epithelium could be a result of MDM-2 amplification, RNA overexpression, or post-translational stabilisation in these tumours. In this respect, it is interesting to note that besides DNA amplification or stabilisation of the protein because of binding to p53, enhanced translation was recently reported as a mechanism of MDM-2 protein accumulation in human tumours.³²

(2) p53 protein expression without MDM-2 protein expression occurred in six cases (four undifferentiated nasopharyngeal carcinoma, one non-keratinising nasopharyngeal carcinoma, and one keratinising nasopharyngeal carcinoma). This pattern suggests deregulation of the self regulating feedback loop which is formed by the interaction between MDM-2 and p53 proteins.³³ With regard to the cases of undifferentiated nasopharyngeal carcinoma, it is possible that other cellular or viral proteins may account for the stabilisation of the p53 protein, as suggested previously.³⁴ Concerning non-keratinising and keratinising nasopharyngeal carcinomas, it is not known whether p53 mutations occur. If they do, it is possible that these mutations are unable to activate the MDM-2 gene. Alternatively, MDM-2 gene deregulation might have occurred in these cases in the absence of p53 gene mutations. It is of interest that MDM-2 protein expression was not found in the absence of p53 protein expression in nasopharyngeal carcinoma. This is in agreement with the finding that the absence of p53 protein will not allow MDM-2 transactivation, with the result that there is no expression of MDM-2.^{21,22,29}

In summary, our study shows that different patterns of concordant or discordant p53/MDM-2 protein expression occur in nasopharyngeal carcinoma, thus suggesting that derangement of the feedback loop that regulates the expression of p53 and MDM-2 may play a role in the pathogenesis of a proportion of cases of nasopharyngeal carcinoma. Further studies at DNA and RNA level are required to clarify the molecular mechanisms which underlie the expression of MDM-2 protein in nasopharyngeal carcinoma.

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