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Expression of *mdm-2* and *p53* protein in transitional cell carcinoma

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Abstract Amplification of the *mdm-2* gene and overexpression of the *mdm-2* protein might inactivate *p53* function, and may have prognostic relevance. The present paper investigated the immunohistochemical overexpression of the *mdm-2* and *p53* proteins in 25 biopsy specimens of transitional cell bladder carcinomas (10 pT1 and 15 pT2 or higher stages). Five cases (20%) showed strong *mdm-2* protein immunoreactivity in more than 5% of the tumor cells; 14 cases (56%) showed *p53* immunoreactivity in more than 20% of the cells, and were considered as overexpressing *p53* protein. Four of the five cases with strong *mdm-2* immunoreactivity did not show *p53* overexpression, and 13 of the 14 cases with *p53* overexpression did not show *mdm-2* immunoreactivity. Our data are consistent with the hypothesis that *p53* overaccumulation (and hence possible *p53* gene mutation) or *mdm-2* overexpression (and hence possible *mdm-2* gene amplification) may mirror two different and possibly complementary gene alterations, which might finally interfere with the control of cell proliferation and apoptosis. In this perspective, evaluation of the combined *mdm-2*/*p53* protein phenotype in human bladder carcinomas could have prognostic relevance and give us better prognostic information than evaluation of the *p53* protein alone.

Key words *mdm-2* protein · Immunohistochemistry
Bladder carcinoma · *p53* protein

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The product of the *mdm-2* gene is a *p53*-binding protein, which can inhibit *p53*-mediated transactivation. It seems that *p53* and *mdm-2* play reciprocal roles in regulating each other [10, 17] and overexpression of the *mdm-2* gene overcomes wild-type *p53*-mediated suppression of transformed cell growth [4]. Overexpression of the *mdm-2* protein might therefore be one of the mechanisms of inactivation of *p53* function [17].

Amplifications of the *mdm-2* gene have been demonstrated in various human neoplasms, including 15–36% of soft tissue sarcomas [2, 8, 12], 14% of osteosarcomas [7], 8–10% of high-grade malignant gliomas [15] and 10% of human breast carcinomas [9]. In other human neoplasms, including Ewing's sarcomas [6], carcinomas of the uterine cervix [5], and myelodysplastic syndromes [14], the *mdm-2* gene is not amplified and seems not to be involved in the neoplastic process.

The relations between *mdm-2* gene amplification and increased expression (both at the mRNA and protein levels) are complex and not completely understood [2]. Tumor may show amplification and overexpression, or amplification without overexpression, or overexpression without amplification [2].

In soft tissue sarcomas overexpression of the *mdm-2* gene seems prognostically relevant [2], and it could be of interest to evaluate the prognostic impact of both *mdm-2* and *p53* overaccumulation in other human neoplasms.

In a previous study we showed that, in breast carcinomas, *mdm-2* gene amplification is associated with *mdm-2* protein overexpression [9] . These data, in keeping with the results of Cordon-Cardo et al. [2] on soft tissue tumors, suggested that *mdm-2* protein immunohistochemistry, even on fixed sections, could be a simple screening method to investigate the *mdm-2* gene status.

Here we show our data on a small series of transitional cell carcinomas of the bladder, which were analyzed for *mdm-2* and *p53* protein expression on formalin-fixed material.

Materials and methods

Materials

Twenty-five bladder transitional cell carcinomas (15 cases of grade 3 stage pT2 or higher, 7 cases of grade 3 stage pT1, 3 cases of grade 2 stage pT1) were received as transurethral biopsy specimens; each tumor was routinely formalin fixed and paraffin embedded. All cases were diagnosed on the basis of H & E-stained sections, classified and graded according to the WHO system [11]. Clinical staging was done according to the UICC TNM scheme [16].

Immunohistochemistry

Sections of the paraffin samples were immunostained for *mdm-2* protein using the IF-2 monoclonal antibody (mAb) [8] (Oncogene Science, Manhasset, N.Y., USA) with microwave pretreatment as previously described [1, 9]. The IF-2 mAb was used at 1:100 dilution for 12 h at room temperature, followed by the highly sensitive streptABC technique (Duett, Dako, Glostrup, Denmark). Serial paraffin sections were immunostained also for *p53* protein (D07 mAb, Novocastra Laboratories, Newcastle upon Tyne, UK) as previously described [3]. Unrelated mAbs of the same IgG isotype were used as negative controls at similar working dilutions.

All immunostained slides were blindly scored by two observers counting at least 1000 cells for each section. Any cell showing nuclear immunoreactivity for the above antibodies was scored as positive, and the percentages of positive cells was recorded for each case. For statistical analysis *mdm-2* and *p53* immunoreactivity were scored as positive if the percentages of stained cell were >5 and ≥ 20, respectively.

Statistical procedure

The association between the *mdm-2* and *p53* labeling indexes were tested for association using Fisher's exact test and the chi-square test using Microstat statistical software run on an Olivetti 386 personal computer (Olivetti, Ivrea, Italy).

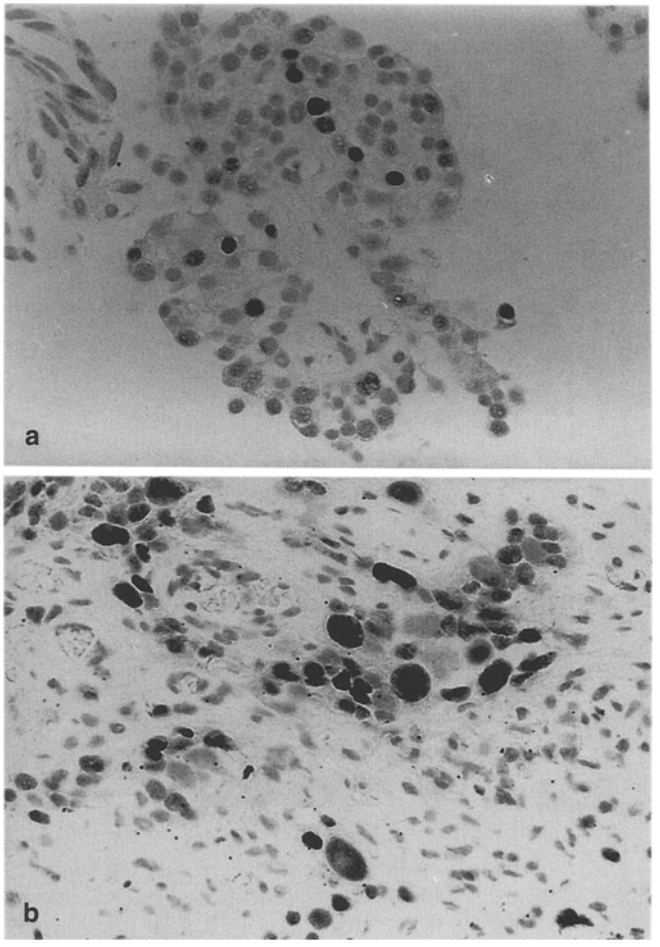

Fig. 1 a, b *mdm-2* protein immunohistochemical expression in two cases of transitional bladder carcinoma, with low (less than 2% of the cells, **a**) and high (more than 70%, **b**) reactivity. Immunoperoxidase on paraffin sections, streptABC technique with light hematoxylin counterstain, original magnification ×400

Results

mdm-2 immunoreactive cells were seen in 10 (50%) cases. Immunoreactivity was always nuclear with some degree of faint cytoplasmic staining. The percentage of immunoreactive nuclei ranged from less than 1% to more than 70%, and strong *mdm-2* immunoreactivity (nuclear labeling in $\geq 5\%$ of tumor cells) was seen in 5 (20%) cases (Fig. 1). Four of the cases with strong *mdm-2* immunoreactivity were grade 3 tumors infiltrating the muscular wall of the bladder (pT2 or higher stage), and one case was a grade 2 pT1 tumor (Table 1).

p53 immunoreactivity was seen in 19 (75%) cases. Immunoreactivity was always nuclear, and the percentage of immunoreactive cells ranged from less than 1% to more then 70%. Strong *p53* immunoreactivity (more than 20% of reactive cells) was seen in 14 cases (56%), which were considered as overexpressing *p53* protein. Nine of the cases with *p53* overexpression were grade 3 tumors infiltrating the muscular wall of the bladder, three cases were grade 3 pT1 tumors (one with squamous metaplasia), and two were grade 2 pT1 tumors (Table 1).

Four of the five cases with strong *mdm-2* immunoreactivity did not show *p53* overexpression, and 13 of the 14 cases with *p53* overexpression did not show strong *mdm-2* immunoreactivity (Table 2).

Discussion

The present study shows that 5/25 (20%) cases of bladder carcinoma show *mdm-2* protein immunohistochemical reactivity, and that there is a trend toward an inverse association between *mdm-2* immunoreactivity and *p53* overexpression.

In a previous study of ours on a series of breast carcinomas [9], we demonstrated that *mdm-2* gene amplification and *mdm-2* protein expression are strictly associated, in keeping with previous results on protein expression in cell cultures and human sarcomas [8, 13], and mRNA expression in high-grade astrocytic tumors [15].

Table 1 *p53* and *mdm-2* immunoreactivity in the series of bladder carcinomas

Case No.	Grade	Stage[a]	*p53* IR[b]	*mdm-2* IR[c]
1	3	pT1	++	0
2	3	pT2 or >	++	0
3	3	pT2 or >	−	0
4	3	pT2 or >	+	0
5	3[d]	pT1	+	0
6	3	pT2 or >	+	0
7	3	pT2 or >	+	0
8	3	pT1	−	0
9	3	pT2 or >	−	0
10	2	pT1	−	0
11	3	pT1	++	0
12	3	pT2 or >	+	0
13	3	pT2 or >	++	1
14	3	pT2 or >	++	1
15	3	pT2 or >	−	1
16	3	pT1	−	1
17	2	pT1	++	2
18	3	pT2 or >	++	2
19	2	pT1	+	3
20	2	pT1	−	3
21	3	pT2 or >	−	5
22	3	pT2 or >	−	10
23	2[d]	pT1	−	20
24	3	pT2 or >	++	30
25	3	pT2 or >	−	90

[a] Pathological stage according to UICC; for pT2 cases the level of bladder wall infiltration could not be determined with certainty on the biopsy, and therefore higher stages could possibly be included
[b] *p53* immunoreactivity was scored as follows: − = negative immunostaining or less than 5% of reactive nuclei, + = from 5 to 20% of reactive nuclei, ++ = more than 20% of reactive nuclei
[c] *mdm-2* protein immunostaining is reported as the percentage of reactive nuclei
[d] Cases with squamous metaplasia

Table 2 Association between *mdm-2* protein overexpression and *p53* overaccumulation (chi-square test without continuity correction factor, $P=0.069$; Fisher's exact test, $P=0.095$; *mdm-2* IR < 5% = *mdm-2* protein immunoreactivity in less than 5% of cells; *mdm-2* IR ≥ 5% = *mdm-2* protein immunoreactivity in 5% or more of cells; *p53* IR < 20% = *p53*/DO7 immunoreactivity in less than 20% of cells; *p53* IR ≥ 20% = *p53*/DO7 immunoreactivity in 20% or more of cells)

	mdm-2 IR < 5%	*mdm-2* IR ≥ 5%	Total
p53 IR < 20%	7	4	11
p53 IR ≥ 20%	13	1	14
Total	20	5	25

It is tempting to hypothesize that the immunohistochemical demonstration of *mdm-2* immunoreactivity in this series of bladder carcinomas could be considered as an indirect sign of possible *mdm-2* gene amplification. Further studies are required to elucidate whether *mdm-2* gene amplification really occurs in bladder carcinomas and whether amplification is really associated with overexpression. Other mechanisms may in fact be responsible for sustained levels of *mdm-2* protein in the absence of amplification, such as chromosomal translocations or mutations which could increase the level of *mdm-2* protein, or post-translational mechanisms.

In the present small series of bladder carcinomas, there is a trend toward an inverse association between strong *mdm-2* protein immunoreactivity and lack of *p53* protein overexpression. Four of five cases with *mdm-2* were devoid of *p53* overaccumulation, and hence possibly devoid of *p53* gene mutations; 13 of 14 cases with *p53* overaccumulation were devoid of *mdm-2* overexpression, and hence possibly devoid of *mdm-2* gene amplification. It could be tempting to hypothesize that our immunohistochemical results on *mdm-2* and *p53* proteins may mirror two gene alterations (amplification and mutation, respectively, for *mdm-2* and *p53* genes), which finally impair the control of cell proliferation and apoptosis. These results on bladder carcinomas could be similar to the those obtained in soft tissue sarcomas, where *mdm-2* amplification and *p53* mutation seem mutually exclusive [12]. Further studies on larger series of bladder tumors with immunohistochemical and genetic data are warranted to verify this hypothesis.

Regardless of the complexities of the relations between the above two genes and gene products, it has been suggested that *mdm-2* and *p53* protein immunohistochemistry might be simple tests to investigate the alterations of the same metabolic pathway in relation to clinical outcome of the patients [2]. In this perspective, evaluation of the combined *mdm-2*/*p53* protein phenotype in human bladder carcinomas could give us better prognostic information than evaluation of the *p53* protein alone.

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