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Expression of Skp2 and p27KIP1 in naevi and malignant melanoma of the skin and its relation to clinical outcome

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Summary. Skp2 (S-phase kinase associated protein 2) controls progression from G- to S-phase by promoting the proteolysis of the cyclin dependent kinase inhibitor p27KIP1. Despite the fact that a p27KIP1 decrease has been documented in melanoma progression, the role of Skp2 in these tumours is unknown. We therefore examined by immunohistochemistry the expression of Skp2, p27KIP1 and Ki-67 in 10 naevi (Ns), 15 superficial spreading melanomas (SSMs), 10 nodular melanomas (NMs) and 14 melanoma metastases (Ms). Nuclear Skp2 expression augmented with increasing malignancy (Ns: 1.4%, SSMs: 5.6%, NMs: 17.3%, Ms: 19.1%). In all tumours nuclear Skp2 expression correlated with Ki-67 (p=0.024) and inversely with p27KIP1 (p=0.007). A cytoplasmic reaction for Skp2 was also observed in most tumours and its expression decreased from Ns (12.3%) to SSMs (7.9%) and NMs (4.5%). In contrast, Ms showed an increase of cytoplasmic Skp2 (11.9%) that correlated with its nuclear expression (p=0.016). While nuclear Skp2 expression correlated with the pT-level (p=0.023), Clark-level (p=0.023) and Breslow index (p=0.019), the cytoplasmic Skp2 expression might be of biological significance only in NMs since it correlated with tumour depth (p=0.02) and pT-level (p=0.025). Our data suggests that Skp2 could contribute to melanoma progression. This is further highlighted by the fact that vertical growth phase (VGP) melanomas show significant higher nuclear Skp2 expressions when compared with the harmless radial growth phase (RGP) (p=0.047). Also nuclear Skp2 expression correlates with a reduced survival time (p=0.025) in melanoma.

Key words: Melanoma, Naevi, Cell cycle, Skp2, p27KIP1

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Introduction

Loss of p27KIP1 contributes to malignant transformation of cells and has been demonstrated in a large variety of tumours (Lloyd et al., 1999). Skp2 plays an important role in the cell cycle promoting the entry into G1 phase by degradation of p27KIP1 resulting in an activation of cyclin E allowing the cell to enter the S phase (Bloom and Pagano, 2003). A specific association between the F-box protein Skp2 (S-phase kinase associated protein 2) and p27KIP1 has been observed during the G1-S phase and is essential for the SCF (Skp, cullin, F-box receptor) directed ubiquitination and subsequent proteolysis of p27KIP1 (Carrano et al., 1999). Stimulation of quiescent cells with growth factors induces the expression of Skp2 in late G1 phase (Wirbelauer et al., 2000; Carrano and Pagano, 2001) and over-expression of Skp2 results in cell cycle progression (Carrano and Pagano, 2001). Skp2 promotes S phase entry in serum-starved cells by accumulation of cyclin A, cyclin E and cyclin dependent kinase 2 (CDK2) activation (Vlach et al., 1997; Sutterluty et al., 1999). Skp2 mediated ubiquination of p27KIP1 requires its phosphorylation at threonine-187 in the nucleus (Tsvetkov et al., 1999), which results from its interaction with CDK2 (Vlach et al., 1997). Furthermore, Skp2 has been identified as target of cell adhesion dependent signalling (Carrano and Pagano, 2001) and it has been suggested that an over-expression of Skp2 represents a growth advantage allowing proliferation in the absence of cell adhesion (Carrano and Pagano, 2001; Bloom and Pagano, 2003).

In malignant melanoma of the skin reduced p27KIP1 levels correlate with tumour progression (Florenes et al., 1998). However, the mechanism of down-regulation of p27KIP1 in melanoma remains largely unknown (Woenckhaus et al., 2004). Numerous studies found high levels of Skp2 in advanced cancers (e.g. oral squamous, gastric, small cell lung and colorectal cancers) and also found increased Skp2 levels to correlate inversely with p27KIP1, suggesting a role for Skp2 mediated degradation of the tumour suppressor protein p27KIP1

in cancer (Gstaiger et al., 2001; Hershko et al., 2001; Kudo et al., 2001; Masuda et al., 2002; Yokoi et al., 2002). An increased Skp2 expression has been correlated to the grade of differentiation and prognosis in oral squamous cell carcinoma (Gstaiger et al., 2001; Kudo et al., 2001) and lymphomas (Latres et al., 2001). Furthermore, the region 5p13 where Skp2 is located (Demetrick et al., 1996) is frequently amplified in small cell lung cancers (Yokoi et al., 2002). Thus, deregulation of Skp2 resulting in its over-expression could contribute to reduction of p27KIP1 and to neoplastic transformation (Bloom and Pagano, 2003). Therefore, and because the relation between p27KIP1 and Skp2 in melanoma is currently unknown, we studied primary melanomas, melanoma metastases and naevi for immunohistochemical expression of Skp2 and p27KIP1. The proliferation was assessed by staining for Ki-67. These results were also compared to clinical outcome and parameters of tumour progression.

Materials and methods

Patients and tumours

Patient data and tissue were obtained and used after advice from the Medical Ethics Committee of the University of Greifswald in accordance with the declaration of Helsinki and the International Conference of Harmonisation – Good Clinical Practice. The anonymity of the patients investigated was preserved corresponding to rules of data protection of the Human Medical Faculty Greifswald and the county Mecklenburg-Vorpommern.

Melanomas were classified as stated elsewhere (Poetsch et al., 1998; Woenckhaus et al., 2003) and included a wide range of tumour thickness (Breslow index measured in millimetre from the outermost granular layer across the tumour in its thickest part) and Clark levels. The latest TNM edition (Sobin and Wittekind, 2002), without sub-classification between ulcerated and non-ulcerated tumours was used for evaluation of the pT-level (Woenckhaus et al., 2004). 49 paraffin embedded melanocytic tumours were investigated: 10 naevi from sun exposed sites (1 epidermal, 5 compound and 4 dermal naevi), 15 superficial spreading melanomas (SSMs), 10 nodular melanomas (NMs) and 14 melanoma metastases (Ms) including 12 lymph node metastases (M) and 2 dermal and subcutaneous skin metastases (M3 and M4). The tumours belonged to 33 patients and follow-up data was available from 25 patients ranging from 12 to 96 months (mean 43.9) (Tables 2, 3). Radial growth phase (RGP) melanomas and vertical growth phase (VGP) melanomas were defined according to the criteria given by Elder et al. (1996).

Immunohistochemistry

Serial sections were cut at 4 µm with a rotation microtome (Microm, Type HM 335E, Walldorf, Germany) and mounted on glass slides coated with 3-aminopropyltriethoxy-silane (Sigma-Aldrich, Taufkirchen, Germany). The first section of each series was stained with hematoxylin-eosin (H&E) and the subsequent sections were stained immunohistochemically. Prior to immunohistochemistry, sections were subjected to heat (96-99 °C) induced epitope retrieval in TEC (tris-base, EDTA and tri-sodiumcitrate) buffer (pH 7.8) for subsequent detection of p27KIP1 and Ki-67 or in EDTA buffer (pH 8.0) for subsequent detection of Skp2. No preblocking was performed. Incubation was performed with a monoclonal antibody against p27KIP1 (1:20; Novocastra, Newcastle upon Tyne, UK) and Ki-67 (1:50, DakoCytomation, Hamburg, Germany) and with a polyclonal antibody against Skp2, reacting to amino acids 1-435 representing full length Skp2 p45 of human origin (1:50; Santa Cruz Biotechnologies, Heidelberg, Germany) for 60 minutes at room temperature. The immunohistochemical reaction was visualised with the ChemMate detection kit (DakoCytomation, Hamburg, Germany) as indicated by the manufacturer. Normal mouse or rabbit serum containing mixed immunoglobulins at a concentration approximating that of primary antibodies was used as negative control. Internal positive controls were present in all sections and consisted either of epidermal keratinocytes or lymphocytes.

Scoring

Nuclear staining was independently scored by two observers (C. W. and J. G.) with a morphometry system for quantitative evaluation. The system was set up by a Zeiss microscope (Axioskop, Jena, Germany), a digital video camera (C4742-95, Hamamatsu, Japan) and a Macintosh computer (Macintosh Power G3, Apple Computer Inc, USA) using Openlab software (Improvision, Coventry, UK). A minimum of 600 melanocytes, which were stained for the corresponding proteins, was counted per sample. At least 10 microscopic high power fields per sample were analysed. To assure the evaluation of melanocytes, immunohistochemically stained slides were always compared to the respective H&E stained section. Immunoreactive score (IRS) was assessed as follows: the number of positive cells in each randomly selected field was determined and expressed as percentage of the total number of tumour cells. When the difference in scoring between each observer was below 10%, the mean value was used and when above 10%, the measurements were repeated twice and the mean values were employed. Staining for p27KIP1 was restricted to the nuclei of tumour cells, while Skp2 was present in the nuclei and the cytoplasm of melanocytes.

Statistical analysis

Statistical evaluation was performed using the

statistical software package SSPS version 12.0.1 (SPSS GmbH Software, Munich, Germany). The results were evaluated using standard statistical methods: t-test, Spearman rank correlation coefficient and one-way ANOVA. $p<0.05$ was considered statistically significant.

Table 1. Immunohistochemical expression of Ki-67, p27KIP1 (p-27) and Skp2 in naevi.

TUMOUR	ki-67	p-27	Skp2 NUCLEAR	Skp2 CYTOPLASMIC
N1	0.5	13.0	0.9	23.2
N2	0.5	23.9	2.2	12.9
N3	0.9	12.4	0.8	9.4
N4	0.7	14.9	0.9	14.5
N5	1.8	9.3	0.5	6.79
N6	1.1	30.1	2.3	7.8
N7	1.0	16.7	1.5	11.9
N8	1.1	14.6	2.8	14.9
N9	2.1	6.7	0.7	13.7
N10	4.5	12.2	1.7	7.8

N: naevus; Ki-67: percentage of positive nuclei; Skp2 nuclear: percentage of melanocytes with positive nuclei for Skp2; Skp2 cytoplasmic: percentage of melanocytes with a cytoplasmic stain for Skp2.

Results

Immunohistochemistry

Melanocytes and epidermal cells showed a nuclear reaction for Skp2 and p27KIP1. While mostly basal keratinocytes demonstrated a nuclear Skp2 expression, p27KIP1 was detectable in the apical keratinocytes (Fig. 1). In normal skin single melanocytes of basal epidermis occasionally showed a granular cytoplasmic staining for Skp2 or a strong homgeneous nuclear reaction for p27KIP1. Positive controls also consisted in lymphocytes of the follicle centre which showed a nuclear reaction for Skp2, while perifollicular lymphocytes strongly expressed p27KIP1 (Fig. 1). In one NM and one M a cytoplasmic stain for Skp2 was completely absent. The immunohistochemical expressions of all proteins investigated are listed in detail in table 1-3, mean values and standard deviations are shown in box plots (Fig. 2A-D).

Table 2. Immunohistochemical expression of Ki-67, p27KIP1 (p-27) and Skp2 in superficial spreading melanomas (SSMs) and nodular melanomas (NMs).

TUMOUR	BRESLOW	CLARK LEVEL	pT	FOLLOW-UP (months)	Ki-67	p-27	Skp2 NUCLEAR	Skp2 CYTOPLASMIC
SSM1 (RGP)	0.02	I	Is	NED60	4.6	6.4	8.12	13.8
SSM2 (RGP)	0.4	II	1a	LTF	10.8	8.3	3.2	17.2
SSM3 (RGP)	0.5	II	1a	NED12	11.5	1.2	12.7	2
SSM4 (RGP)	0.45	II	1a	NED18	10.3	6.9	4.2	11.5
SSM12 (RGP)	0.01	I	Is	NED12	22.7	1.8	0.3	4.1
SSM13 (RGP)	0.03	I	Is	NED96	4.5	6.8	0.7	7.4
SSM14 (RGP)	0.02	I	Is	LTF	8.2	7.5	1.9	1.3
SSM5 (VGP)	1	III	1a	LTF	21.5	2.4	3.7	7.8
SSM6 (VGP)	1.0	III	1a	LTF	9.1	2.0	1.8	3.7
SSM7 (VGP)	1.4	III	2a	LTF	18.5	4.2	5	12.3
SSM8 (VGP)	1.2	III	2a	NED60	15.2	2.7	6.5	12.9
SSM9 (VGP)	1.1	III	2b	AWD24	17.1	2.6	0.05	2.3
SSM10 (VGP)	1.6	III	3a	NED48	7.9	6.2	18.86	1.4
SSM11 (VGP)	2	III	2a	LTF	4.8	1.4	14.6	15.12
SSM15 (VGP)	0.6	III	1a	LTF	15.3	10.8	2.1	5.8
NM1	2	IV	2a	LTF	4.7	6.1	11.78	9.2
NM2	2	III	2a	AWD96	5.9	6.0	0.38	3.7
NM3	3.5	IV	3b	DOD48	3.,5	1.3	17.5	1.5
NM4	3.5	IV	3a	DOD13	7.6	6.4	48.2	4
NM5	5	V	4a	LTF	14.4	2.0	32.4	13.2
NM6	5	IV	4a	LTF	8.9	1.7	2.8	2.4
NM7	5	IV	4a	LTF	19.7	4.1	0.5	3
NM8	6	V	4a	DOD60	7.5	6.0	39.7	3.2
NM9	1.7	III	2a	LTF	9.1	3.2	16.7	4.9
NM10	6.52	V	4a	DOD17	32.2	3.6	20.9	0

SSM: superficial spreading melanoma; NM: nodular melanoma; RGP: radial growth phase; VGP: vertical (Elder et al., 1996) growth phase; Breslow: Breslow index (tumour thickness in millimetre); pT: tumour extension according to the latest TNM classification (Sobin and Wittekind, 2002); follow-up: clinical follow up in month; AWD: alive with disease; NED: no evidence of disease; DOD: dead of disease; LTF: lost to follow-up; Ki-67: percentage of positive nuclei; Skp2 nuclear: percentage of melanocytes with positive nuclei for Skp2; Skp2 cytoplasmic: percentage of melanocytes with a cytoplasmic stain for Skp2.

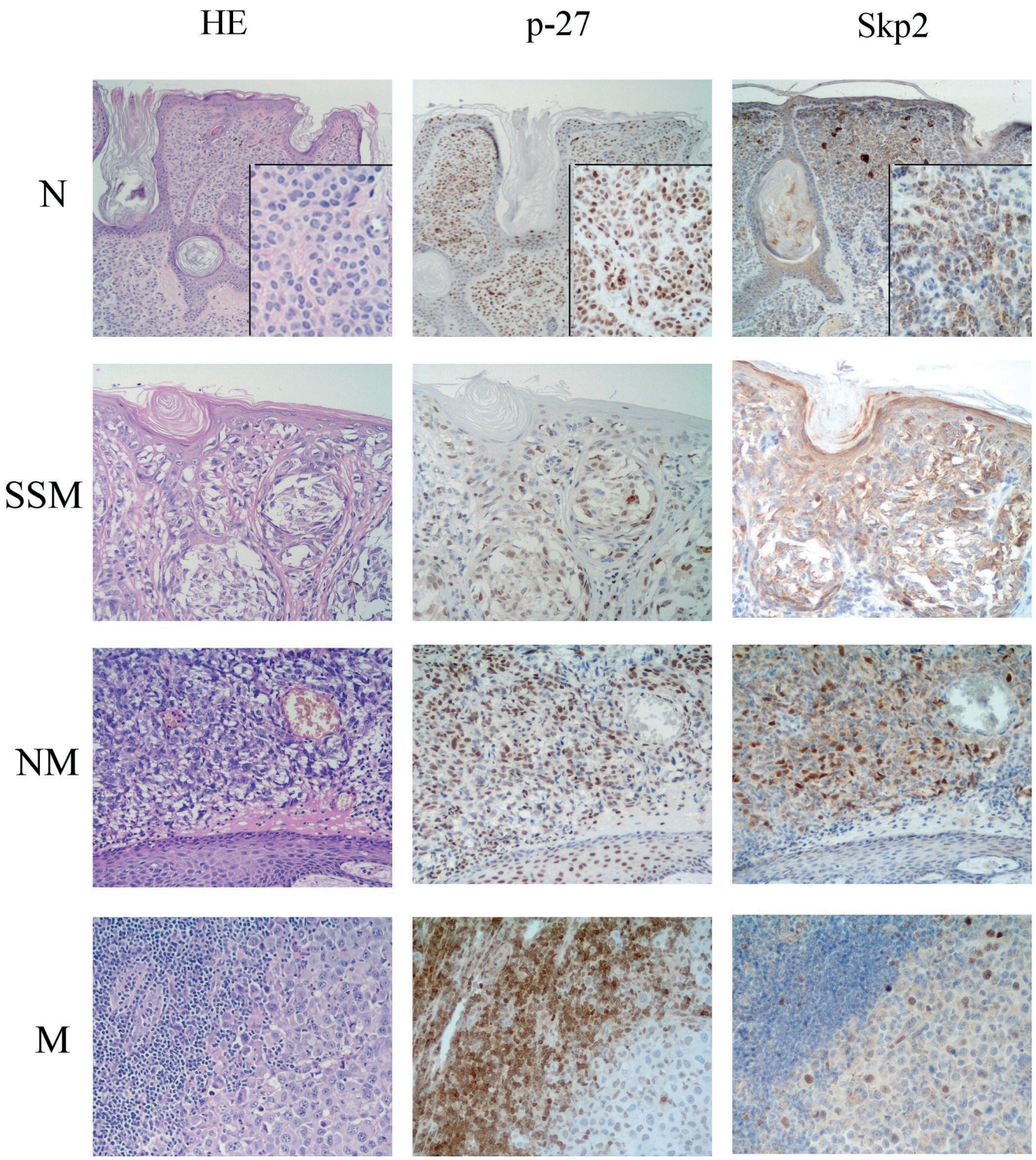

Fig. 1. From the left to the right: H&E stain, immunohistochemical expression of p27KIP1 (p-27) and Skp2. From the top to the bottom: dermal naevus (10x), superficial spreading melanoma (20x), nodular melanoma and melanoma lymph node metastasis (both 20x). Inset: larger amplification (40x) in the naevus showing nuclear expression of p27KIP1 (p-27) and cytoplasmic stain for Skp2. Note that apical keratinocytes are positive for p27KIP1 and coarse melanin pigment is contrasting with the immunohistochemical reaction.

Statistical evaluation

The nuclear expression of Skp2 (Skp2 nuc) and Ki-67 significantly increased (both $p<0.001$, one-way ANOVA), whereas that of p27KIP1 significantly decreased ($p<0.001$, one-way ANOVA) when comparing Ns, the different histogenetic subtypes of melanoma (SSM, NM) and the Ms (Fig. 2A,B,D). Mean values for Ki-67 and Skp2 nuc expression were significantly higher in SSMs when compared to Ns, while expression of p27KIP1 was significantly lower (Fig. 2A,B,D). In Ns the cytoplasmic expression of Skp2 (Skp2 cyto) was significantly elevated when compared to SSMs and NMs and when comparing Ms with NMs (see Fig. 2C).

The significant correlations between the nuclear expression of Skp2, p27KIP1, Ki-67 and parameters of tumour progression are summarised in Table 4. In all lesions nuclear Skp2 expression correlated with expression of Ki-67 and inversely with the expression of p27KIP1. All parameters of tumour progression also correlated with the nuclear Skp2 expression (Table 4). Interestingly, the cytoplasmic expression of Skp2 in Ms correlated inversely with nuclear Skp2 values only in these tumours ($p=0.016$, $r_s=-0.63$). In NMs an increase of cytoplasmic Skp2 expression correlated with the pT

Table 3. Immunohistochemical expression of Ki-67, p27KIP1 (p-27) and Skp2 in metastases (Ms).

TUMOUR	FOLLOW-UP (months)	Ki-67	p-27	Skp2 NUCLEAR	Skp2 CYTOPLASMIC
M1	DOD60	42.8	5.7	0.9	34.6
M2	DOD60	22.4	5.0	17.2	1.3
M3	LTF	8.3	1.3	9.6	25.6
M4	DOD13	36.9	5.6	41.8	0
M5	DOD48	16.8	4.9	7.8	5.46
M6	DOD48	41.4	2.4	18.2	26.4
M7	LTF	8.7	1,4	39.8	1.3
M8	AWD16	13.3	1.6	35.2	3
M9	DOD60	6.9	4.0	16.7	23.8
M10	DOD60	11.9	8.4	21.9	18.1
M11	DOD17	30.6	2.1	19.7	2.1
M12	LTF	6.9	2.6	3.9	23.4
M13	DOD60	18.6	2.0	5.1	1.7
M14	DOD48	36.7	2.3	30.2	0.7

M: metastasis; follow-up: clinical follow up in month; AWD: alive with disease; NED: no evidence of disease; DOD; dead of disease; LTF: lost to follow-up; Ki-67: percentage of positive nuclei; Skp2 nuclear: percentage of melanocytes with positive nuclei for Skp2; Skp2 cytoplasmic: percentage of melanocytes with a cytoplasmic stain for Skp2.

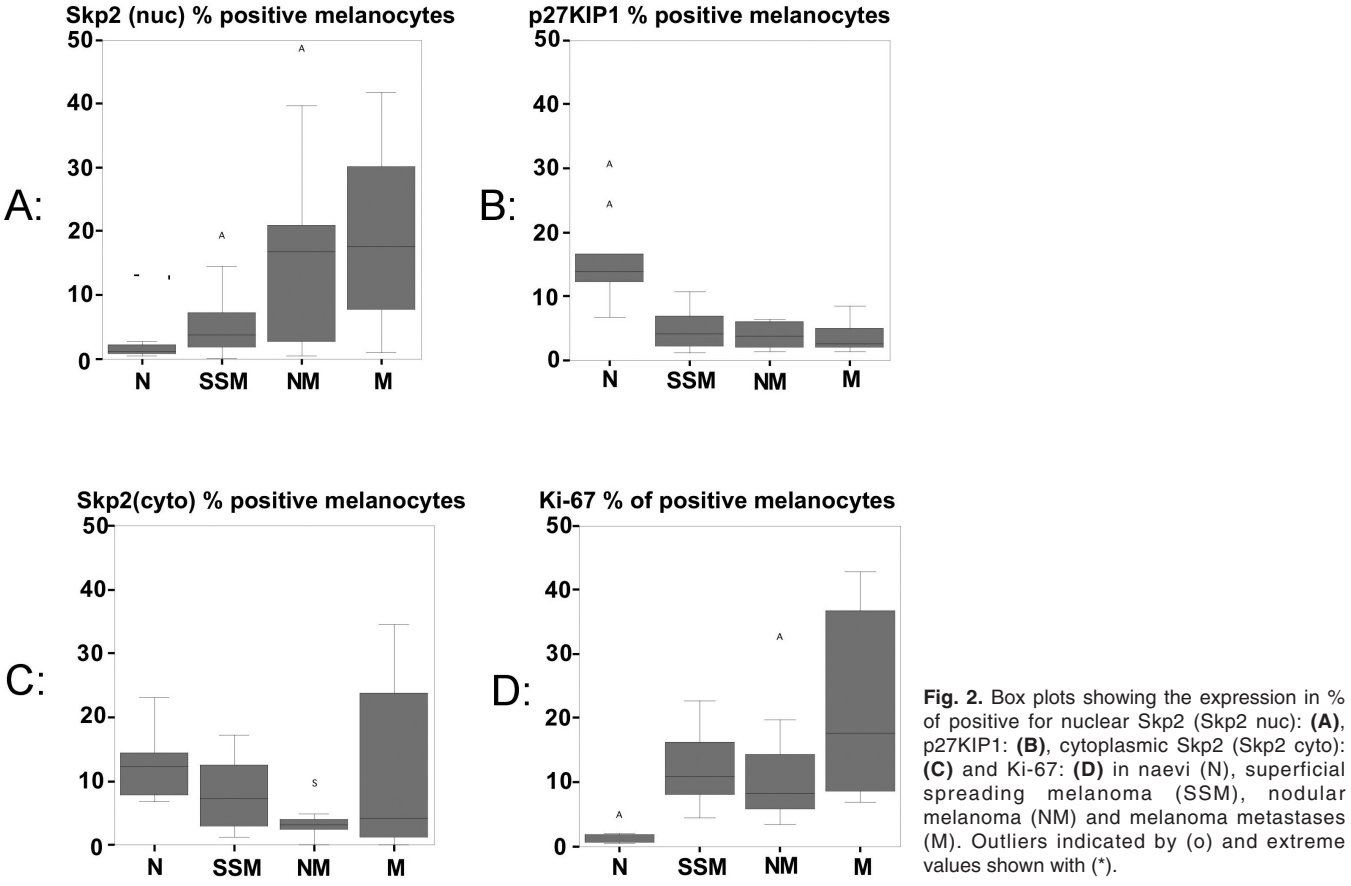

Fig. 2. Box plots showing the expression in % of positive for nuclear Skp2 (Skp2 nuc): **(A)**, p27KIP1: **(B)**, cytoplasmic Skp2 (Skp2 cyto): **(C)** and Ki-67: **(D)** in naevi (N), superficial spreading melanoma (SSM), nodular melanoma (NM) and melanoma metastases (M). Outliers indicated by (o) and extreme values shown with (*).

level (p=0.025, r_s=-0.79) and with the Breslow index (p=0.02, r_s=-0.75). The nuclear expression of Skp2 significantly increased in VGP when compared to RGP melanomas (Table 5). For all other proteins no significant differences were observed when comparing the less aggressive melanomas (RGP) with the tumours harbouring metastatic potential (VGP). Clinical outcome significantly declined with high nuclear Skp2 expression and Ki-67 expression (Table 5).

Discussion

Regulation of p27KIP1 is an essential step in the pathway that links mitogenic signals to the cell cycle progression and provides critical control of the cell cycle commitment (Philipp-Staheli et al., 2001). Cell cycle progression from G to S-phase is driven by a decrease of p27KIP1 and an increase of Skp2, which targets p27KIP1 for degradation (Bloom and Pagano, 2003). Therefore we compared the immunohistochemical expression of Skp2 and p27KIP1 in order to investigate the relation between both proteins and to evaluate if Skp2 expression could contribute to p27KIP1 down-regulation in melanoma. In this study, low expression of nuclear Skp2 in naevi compared to melanomas and in melanomas of low malignancy when compared to advanced tumours or metastases is in line with studies demonstrating an oncogenic potential of Skp2. In cultured fibroblasts increased levels of Skp2 in interaction with H-Ras cause malignant transformation (Gstaiger et al., 2001) and lymphoma genesis in mice (Latres et al., 2001). Additionally, the correlation between nuclear Skp2 and p27KIP1 expression in naevi and melanomas (p=0.007) found here shows that Skp2 could be responsible for p27KIP1 degradation in both benign and malignant melanocytes.

Reduced levels of p27KIP1 have been associated with malignancy and poor prognosis in a variety of other cancers (Lloyd et al., 1999) and an inverse relation between Skp2 and p27KIP1 has also been observed in prostate (Ben-Izhak et al., 2003), oral squamous (Gstaiger et al., 2001; Kudo et al., 2001) and colorectal carcinomas (Hershko et al., 2001) suggesting an important role for Skp2 in p27KIP1 degradation and tumour progression. High levels of p27KIP1 in naevi as in the present study could be sustained by a low nuclear Skp2 concentration, which could additionally prevent malignant transformation. On the other hand the stepwise increase of nuclear Skp2 expression correlates with tumour progression in melanomas. The altered balance of Skp2 and p27KIP1 also affects the cell cycle in melanoma as shown by the increased proliferation correlating with low p27KIP1 and high Skp2 levels (p=0.007 and p=0.024 respectively). However, high levels of nuclear Skp2 in melanomas do not solely reflect an increase of proliferation, but also contribute significantly to malignancy in these tumours. The relative small portion of tumour cells with markedly increased levels of Skp2 might reflect more aggressive clones. Among all proteins investigated immunohistochemically in this study, the nuclear Skp2 expression exclusively discriminates between the radial growth non-metastasising and the vertical growth melanomas having a far worse prognosis (Elder et al., 1996). From the finding that Skp2 expression is cell-adhesion independent in transformed cells in contrast to normal cells (Carrano and Pagano, 2001) it seems likely that nuclear Skp2 expression could also directly facilitate melanoma growth and increase the metastatic potential by adhesion independent growth. This hypothesis is underlined by the fact that in mammary cancer cells an inhibition of Skp2 leads to a decrease of adhesion independent growth (Signoretti et al., 2002). The relevance of Skp2 during melanoma progression is also shown in the present study by its possible prognostic significance. Even though a relative small number of patients were investigated, an increased expression of nuclear Skp2 correlates with a reduced survival time (p=0.025), which is in line with other cancer types (Kudo et al., 2001; Masuda et al., 2002; Oliveira et al., 2003; Seki et al., 2003).

The anaphase-promoting complexes containing the protein Cdh1 (APCCDH1) that mediates Skp2 poly-

Table 4. Significant correlations (Spearman rank correlation r_s=correlation coefficient) between nuclear Skp2 (Skp2 nuc) expression and Ki-67, p27KIP1 and parameters of tumour progression (pT-, Breslow- and Clark-level).

Skp2 NUC STAINING CORRELATES WITH:	r_s	Spearmann-rank
Ki-67	+0.32	p=0.024
p27KIP1	-0.38	p=0.007
pT-level	+0.46	p=0.023
Breslow-level	+0.47	p=0.019
Clark-level	+0.45	p=0.026

Table 5. Differences between radial growth (RGP) and vertical growth (VGP) melanomas and differences between patients alive and patients dead of disease (DOD) expressing nuclear Skp2 (Skp2 nuc), p27KIP1, cytoplasmic Skp2 (Skp2 cyto) and Ki-67.

	% OF POSITIVE STAINED CELLS mean±SD		
	RGP (n=7)	VGP (n=18)	t-test
Skp2 nuc	4.4±4.49	12.4±13.93	p=0.047
P27KIP1	5.6±2.84	4.0±2.47	p=0.198
Skp2 cyto	8.2±6.15	5.5±4.45	p=0.239
Ki-67	10.4±6.14	12.4±7.47	p=0.533
	ALIVE (n=11)	DOD (n=13)	
Skp2 nuc	9.5±10.94	22.2±14.33	p=0.025
P27KIP1	3.9±2.45	4.5±1.97	p=0.557
Skp2 cyto	8.2±6.15	5.5±4.45	p=0.239
Ki-67	10.6±6.09	24.0±13.41	p=0.005

ubiquitination and subsequent degradation (Bashir et al., 2004; Wei et al., 2004) regulate the expression of Skp2. Possible mechanisms leading to an over-expression of Skp2 in malignant tumours could be a deregulated APC^{CDH1} pathway (Bashir et al., 2004) or an increase of Skp2 gene copy numbers (Yokoi et al., 2002; Zhu et al., 2004). Cytoplasmic translocation of Skp2 could be another regulatory mechanism. This has been described for p27KIP1 where the murine protein Jab1 causes its cytoplasmic localisation and degradation (Tomoda et al., 1999). Skp2 is physiologically present in cytoplasm at low concentrations. With increasing expression Skp2 moves with the SCF core protein (Skp-Cullin-F-box protein) to the nucleus where it promotes the degradation of p27KIP1 (Penin et al., 2002). Besides cytoplpasmic localisation in naevi and melanomas in this study, the cytoplasmic expression of Skp2 has been shown in mesenchymal (Penin et al., 2002), epithelial (Dowen et al., 2003), neural (Schiffer et al., 2002) and lymphatic tumours (Lim et al., 2002). Even though, Skp2 and p27KIP1 complex in the cytoplasm of diffuse large b-cell lymphomas, little is known about the function of Skp2 in this cellular compartment (Lim et al., 2002).

The elevated cytoplasmic expression of Skp2 in naevi could correspond to a low nuclear Skp2 concentration. However, in Ns, SSMs and NMs the nuclear Skp2 expression did not correlate with the Skp2 levels in the cytoplasm. Possibly this could be due to a recently described splice variant of Skp2 which is unable to enter the nucleus (Ganiatsas et al., 2001). Our results in Ms suggest that further genetic changes during tumour progression could alter the nuclear permeability leading to an inverse correlation between cytoplasmic and nuclear Skp2. Interestingly, only in NMs cytoplasmic Skp2 levels correlated with markers of tumour progression (pT level, p=0.025 and Breslow-index, p=0.02). Similar results regarding the histogenetic subtypes of melanoma have been observed for p27KIP1 in NMs (Florenes et al., 1998) and for cyclin A in SSMs (Florenes et al., 2001) which might reflect the peculiarity of these tumours as demonstrated by their specific genetic alterations (Poetsch et al., 2003).

Taken together, we could show that the nuclear over-expression of Skp2 significantly increases with malignancy and could contribute to p27KIP1 degradation. Furthermore Skp2 could serve as prognostic marker in melanoma. The cytoplasmic expression of Skp2 seems to have biological significance in nodular melanomas where it correlates with parameters of tumour progression. Additionally, the nuclear expression of this oncogenic protein characterises the transition from radial to vertical melanoma growth, which could make Skp2 a promising target to control tumour extension.

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