Giornale Italiano di Dermatologia e Venereologia
EDIZIONI MINERVA MEDICA

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Giornale Italiano di Dermatologia e Venereologia 2018 Sep 24
DOI: 10.23736/S0392-0488.18.06145-X

Article type: Original Article

Article first published online: September 24, 2018
Manuscript accepted: September 13, 2018
Manuscript received: July 19, 2018

Immunohistochemical expression and prognostic role of CD10, CD271 and Nestin in primary and recurrent cutaneous melanoma.

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KEYWORDS: Melanoma, in transit metastases, CD10, CD271, Nestin

ABSTRACT

BACKGROUND: CD10, CD271 and Nestin, which are proteins associated with tumor-initiating properties and/or progression potential, have not been specifically studied on malignant melanoma (MM) with cutaneous recurrences.

MATERIALS AND METHODS: We evaluated the expression of of CD10, CD271, and Nestin in 27 tumor samples from 16 patients. These tumor samples corresponded to 6 primary melanomas which developed 11 ITM and 10 primary melanomas without recurrences at 10-year follow-up from specimens obtained from surgical excisions of patients referred to the Unit of Dermatology, Department of Pathophysiology and Transplantation, University of Milan, between 2006 and 2016.

RESULTS: We demonstrated a higher expression of CD271 and Nestin in primary tumors which recurred than control population, Nestin was expressed with significantly higher percentages in primary tumors than recurrences, and CD10 expression was statistically significant correlated with disease-free survival: cases with a lower score recurred lately than cases with higher scores.

CONCLUSIONS: Our preliminary results suggest that CD271 and Nestin can be considered early biomarkers for the development of ITM, Nestin can be useful in differentiating primary MM from cutaneous recurrences, and CD10 is associated with a rapid disease progression and may be considered a potential prognostic marker.

INTRODUCTION

In transit metastases (ITM) are cutaneous or subcutaneous lesions located between the primary site of melanoma and the loco-regional lymph nodes, probably due to spreading of neoplastic cells along lymphatics.

They develop in 5-10% of melanoma patients as visible or palpable lesions usually easily clinically diagnosed (1). According to AJCC 2009 staging classification (2) they are included in stage IIIb

(whitout node involvement), and IIIc (with node involvement) with a 5-year survival rates of 24 and 54% respectively (3).

Their correct identification and immunohistochemical characterization is crucial since they are considered an important risk factor for distant metastases, either metachronous or synchronous (1).

In malignant melanoma (MM) CD10, CD271 and Nestin proteins are variably expressed and associated with tumor-initiating properties and/or progression potential, with consequent prognostic and outcome implications (4, 5, 6).

In particular CD10, also known as neutral endopeptidase, enkephalinase, neprilysin, and common acute lymphoblastic leukemia antigen, is a 90- to 110-kd cell-surface zinc-dependent metalloprotease that inactivates a variety of physiologically active peptides (enkephalin and substance P) which have been demonstrated to suppress tumor progression in melanoma (7).

Previous studies have demonstrated that the genetic and/or epigenic alterations occurring in the multipotent tissue-specific adult stem cells may lead to their malignant transformation into cancer progenitor cells (8).

In addition, markers of melanocytic stem cells have been recently described (9), including Nestin, an intermediate filament which is expressed mainly in the cytoplasm of neuroepithelial stem cells (10) and CD271, also known as nerve growth factor receptor, p75NTR, TNFRSF16, and Gp80-LNGFR, a transmembrane signaling receptor involved in negative cell cycle regulation, specific for neural crest origin (11).

The role of these cellular markers, that may be used in clinical practice easily identified using immunohistochemical staining, in the development of cutaneous metastasis has not been studied so far.

The aim of our study was to compare CD10, CD271, and Nestin expression profiles in primary MMs, their ITM, and a control group of MM which have not metastasized during a follow-up period of at least 10 years, correlating their expression with clinico-pathological variables.

Particularly in this study we aimed to:

- Evaluate their different expression in the metastatic melanoma and in the control group;

- Evaluate their different expression in primary MM and ITM;
- Correlate markers' expression with survival data in the metastatic melanoma group.

MATERIALS AND METHODS

Patients and tissues

We investigated 6 cases of cutaneous malignant melanoma with a consensual diagnosis of at least 3 experienced dermatopathologists which recurred with cutaneous metastases during follow up and 10 cases of cutaneous malignant melanoma which did not show recurrence during a follow-up period of at least 10 years (control population).

All the specimens were obtained from surgical excisions of patients referred to the Unit of Dermatology, Department of Pathophysiology and Transplantation, University of Milan, between 2006 and 2016.

Data regarding age, sex and clinical features, and follow-up of patients were obtained from the computerized medical records.

Additional information included sentinel lymph node biopsy (SLNB) status, years of follow-up, and outcome.

Immunohistochemical evaluation

All the tissue specimens were serially sectioned at 5 µm in thickness.

After deparaffinizing and rehydrating, each tissue section was pretreated, using a retrieval buffer solution pH8 (EDTA) and boiled 3 times for 5 minutes in a pressure cooker according to Cattoretti et al. (12)

Then, each section was incubated with the specific monoclonal antibodies anti Nestin (1:200), (Monoclonal Antibody anti-human Nestin RAT-401), anti CD10 (1:50) (CALLA, Leica) and CD271 (1:200) (NGFR5, Ab1, Neomarkers) at room temperature for 45 minutes, washed with TBS pH 7.6 and incubated in a biotinylated goat anti-mouse anti-rabbit immunoglobulins (Dako REAL™,cod.K5005, Dako, Glostrup, Denmark) at room temperature for 30 minutes. After incubation

with the secondary antibody and a new wash with TBS pH 7.6, sections were incubated with streptavidin conjugated to alkaline phoshatase (Dako REAL™,cod.K5005, Dako, Glostrup, Denmark) at room temperature for 30 minutes A red chromogen solution was prepared as indicated by Dako REAL™ datasheet and used as enzyme substrate according to Cordell et al. (13) Finally, each section was counterstained in Mayer's Hematoxylin solution and coverslipped.

Nestin, CD10 and CD271 expression was evaluated by recording percentage of stained cells, with the elaboration of a scoring system (0 = no staining, 1 = staining in less than 25% of neoplastic cells, 2 = staining in between 25 and 50% of neoplastic cells, 3 = staining in between 50 and 75% and 4 = staining in more than 75% of neoplastic cells).

Statistical analysis

Differences in the expression of the three markers have been investigated using the independent samples T-test (MedCalc Software, Acacialaan, Ostend, Belgium). Statistically significant probability values (p) were considered equal to or less than 0.05. The correlation of protein expression to patient survival was evaluated with Cox's proportional-hazards regression, survival curves were constructed using the Kaplan-Meier method (MedCalc Software).

RESULTS

A total of 27 tumor samples from 16 patients were assessed by immunohistochemical analysis for their expression of CD10, CD271, and Nestin.

These tumor samples corresponded to 6 primary MM which developed 11 ITM and 10 primary MM without recurrences at 10-year follow-up.

Between the 6 cases of malignant cutaneous melanoma with cutaneous recurrences during follow up, 2 were male and 4 were female with a median age of 69 (38-92) years.

Of the 10 cases of the control population, 4 patients were male and 6 were female, with a median age of 43 (33-56) years.

Demographic characteristics, main clinical and histopathologic data are summarized in Table 1.

CD10, CD271 and Nestin expression

In our series, all these markers were found to be expressed in all lesions, with varying degrees except for CD271 which was negative in two recurrences.

Expression scores for all the markers are illustrated in Table 2.

We demonstrated a higher expression of CD271 and Nestin in primary tumors which recurred than control population (p=0.02, SE: 11,0180, 95% CI: 3,7020 to 50,9646 and p=0.0001, SE: 5,2068, 95% CI: -38,8342 to -16,4991 respectively) with T-test.

We next investigated the difference of expression of the proteins between primary lesions and recurrences: considering the whole population, Nestin was expressed with significantly higher percentages in primary tumors than recurrences (p=0.03, SE: 7,1399, 95% CI: 1,1482 to 30,9352) with T-test.

Finally, CD10 expression was statistically significant correlated with disease-free survival: cases with a lower score recurred lately than cases with higher scores (p=0.02, HR= 4,2174, 95% CI: 0,5523 to 32,2042).

There was no association between the degree of the markers' expression and other clinic-pathological data such as age, sex or site.

The expression of CD10, CD271, and Nestin was not significantly influenced by TNM staging at the time the tumor sample was taken.

DISCUSSION

Compared with the control group, expression of CD271 and Nestin was significantly increased in primary MMs which recurred, indicating that they can play a role in the development of ITM and interesting they can predict further cutaneous metastatic progression when expressed by tumor cells at the time of diagnosis of melanoma.

Although they have not been specifically studied on MM with cutaneous recurrences, ours results are consistent with previous observations.

In fact CD271 positive melanoma stem cells have shown correlation with a higher metastatic potential and worse prognosis in immunodeficient mice, and to metastases but not prognosis in humans (5, 14).

Recently finding provide new insight in the role of CD271 in DNA repair, drug response and melanoma metastatic process in a CD271-associated signaling network potentially regulated by p53 (15). CD271 would seem not only influence stem cell functions, like tumorigenicity and plasticity, but is also highly expressed in patient-derived cell cultures from melanoma metastases (16).

It was also suggested that Nestin's expression is associated with melanoma invasiveness.

The presence of Nestin in patients' blood was linked with the presence of circulating melanoma tumor cells in a context of metastatic melanoma (17).

According to our data Nestin was the only marker that significantly discriminated primary tumors and ITM, being expressed with higher percentages in primary tumors than recurrences.

This different expression could be useful in case of complex histological differential diagnosis.

We didn't find any significant variation between primary tumors and metastases in CD271 expression, that instead it has been reported to increase in metastatic melanoma to the brain when compared to controls group including also primary MMs (18).

There was no association between CD10, CD271, and Nestin expression and Breslow depth, Clark level, pathologic stage, sentinel lymph node status, and death.

However, after adjusting for the other prognostic factors, CD10 expression correlates with disease-free survival, being higher expression associated with earlier cutaneous recurrences.

The prognostic value of CD10 in melanoma patients has been already evaluated in previous studies which pointed out its association in MM tumor cells with more rapid disease progression, in addition to an increase risk of death, data that our results did not confirmed (4, 19).

In line with our observations Mohamed et al. (5) didn't find any association between CD271-positive melanomas and patient's outcome.

In contrast, Nestin was observed to be associated with poorer clinical outcome and it was considered an important early marker for survival rate in melanoma patients (6, 20).

CONCLUSION

These results suggest that:

- CD271 and Nestin can be considered early biomarkers for the development of ITM;

- Nestin can be useful in differentiating primary MM from cutaneous recurrences;

-CD10 is associated with a rapid disease progression and may be considered a potential prognostic marker for the association of its high expression with low disease-free survival .

Even though they are preliminary results that should be validate by larger sample size, studying the variability of the expression of these markers in the cutaneous metastatic process is interesting particular because it can modify patients' follow-up and it may have therapeutic and/or diagnostic implications.

ACKNOWLEDGEMENTS

Financial support: none.

Conflicts of interests: none.

TABLES

Table 1 – Clinicopathological characteristics of the series

GROUP	MM	CONTROL GROUP
PATIENTS/TUMOR SAMPLES (n)	6	10
MEDIAN AGE (years)	69	43
SEX RATIO (M/F)	2/4	4/6
MEDIAN BRESLOW THICKNESS (mm)	3.3	0.8
TUMOR SUBTYPE		

-NM -SSM	-3 -3	-2 -8
TUMOR SITE -EXTREMITY -TRUNK	 -6 -0	 -7 -3
SLNB -POSITIVE -NEGATIVE	 -2 -4	 -0 -10
MEDIAN DFS (years)	1.8	12
DISEASE-RELATED DEATH (n)	1	0

NM: nodular melanoma, SSM: superficial spreading melanoma , SLNB: sentinel lymph node biopsy,

DFS: disease-free survival.

Table 2 – Expression scores of the markers

Cases with recurrences

	Primary			Recurrences (mean values)		
	CD10	CD271	Nestin	CD10	CD271	Nestin
1	5	50	80	11,5	27,5	77,5
2	30	20	90	50	35	90
3	20	10	90	20	5	90
4	50	50	90	27,5	32,5	90
5	20	10	90	20	20	80
6	30	20	80	50	25	85

Control population

	CD10	CD271	Nestin
1	10	50	50
2	10	50	70
3	5	10	70
4	20	50	70
5	10	50	50
6	5	50	70
7	5	50	50
8	30	50	40
9	10	90	50
10	30	90	70

Figure 1: Melanoma with recurrence, H&E (A), CD10 (B), CD271 (C) and Nestin (D) immunoistochemical stainings (4x). Melanoma without recurrence, H&E (E), CD10 (F), CD271 (G) and Nestin (H) immunohistochemical stainings (4x).

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