

A Study of C-MYC, SOX10 and BCL-2 Proteins Expression in Head and Neck Mucosal Melanomas

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Abstract: *Background:* Head and Neck Mucosal Melanoma (HNMM) is rare, accounting 1% of all melanomas in USA, and 6% in Japan. Melanoma's development is a complex process involving activation of proto-oncogenes and loss of tumor suppressors. *BCL-2* oncogene encodes a family of anti-apoptotic proteins and it is overexpressed in melanomas. On the other hand, proto-oncogene *C-MYC* is a transcriptional factor and plays crucial role both in driving cell proliferation and promoting apoptosis, its overexpression has been associated to melanoma progression. Moreover, oncogene *SOX10* cooperates with other transcription factors to direct the development and differentiation of melanocytes. These three nuclear markers are associated to melanoma's metastatic risk.

Methods: We studied of *BCL-2*; *C-MYC* and *SOX10* proteins in 19 Formalin Fixed Paraffin Embedded cases of HNMM from Yale School of Medicine and 10 cases from University of São Paulo by Alkaline Phosphatase immunohistochemistry technique.

Results: We considered as positive expression when over 25% of tumor cells were immunostained. We observed 27/29 were positive to *BCL-2* and 28/29 cases showed *SOX10* expression (both markers showing immunostaining in over 75% of tumor cells). However, we noticed variable expression for *C-MYC* in 15/29 of HNMM cases.

Conclusions: According to ours results, we suggest that *BCL-2* and *SOX10*, should be good adjuvant biomarkers for HNMM, however lower expression of *C-MYC* has not shown to play main role in the HNMM development, thus further molecular biology studies should corroborate this present study.

Keywords: Mucosal melanoma, C-MYC, SOX10, BCL-2.

INTRODUCTION

The earliest case of mucosal melanoma was mentioned by Weber in 1859, later it was recognized as a specific pathological entity by Lucke in 1869. However, in the English literature, the first mucosal melanoma was reported by Lincoln in 1885 [1-3]. Mucosal melanoma is generally developed in ectodermally derived mucosal tissues such as: sinonasal and oral cavities, nasopharynx, larynx, tracheobronchial tree and upper esophagus [2-6].

Head and Neck Mucosal Melanoma (HNMM) is a very rare and aggressive neoplasm and it accounts 0.3 – 3.8% of all melanomas cases in Unites States [2-8], and 6% in Japan [9]. HNMM has a poor prognosis with an overall 5 year survival rates ranging from 10 – 35% [6-8,10-12], typically it is associated with local lymph node and/or distant metastasis in the early stages of this disease [5,12].

The etiology of HNMM is still unknown, differently from cutaneous counterpart, it arises in photo-protected sites, it exhibits a different profile of cytogenetic alterations [13,14]. Recruiting and activating a number of intracellular signaling pathways implicate in this tumor progression such as: *CKIT*; *PI3K/AKT*; *MAPK*; *Wnt/β-catenin*; *Src*; *Janus kinase*, signal transducers and activators of transcription [2,14].

The *C-MYC* is a proto-oncogene and a nuclear phospho protein, it is associated with DNA rearrangements and amplification contributing to cell proliferation and differentiation and inducing cancers [15-18]. *C-MYC* expression levels have been related to the prognostic of several types of cancer, however its influence in melanoma development and progression is still contradictory [18,19]. This transcription factor is upregulated in human malignancies, and its overexpression promotes the acquisition of a transformed phenotype in cooperation with other cellular oncogenes, although its role in melanomagenesis still not established [20], it is known that *C-MYC* plays a role in *PI3K/Akt* and *TGF-β*

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induced EMT (epithelial-mesenchymal transition), but it is unclear how these signals are interacted and integrated in melanoma metastasis [21].

SOX-10 is a transcription factor that activates upstream of *MITF* expression and synergizes with *MITF* activating downstream targets [22-23] in melanin synthesis, and also in melanomagenesis [24]. It is expressed in migratory neural crest cells, melanoblast and melanocytes, and overexpressed in melanomas [25]. It is present in 31% of melanocytic nevi; 9-43% of primary melanomas; 22-50% of metastatic melanoma [22,26], and it represents a promising target for congenital naevi and melanoma treatment [26].

On the other hand, *BCL-2* a proto-oncogene that prolongs the survival of cells in the absence of required growth factors by blocking apoptosis, even in the presence of apoptotic stimulation [27], and also contributes to neoplastic cell growth [28]. The regulation of *BCL-2* expression is demonstrated to be critical for melanocyte and melanoma survival [29] and it has been attributed to *MITF*, and its overexpression reduced basic apoptosis and sensitivity of melanoma cells for proapoptotic stimuli [30]. However, the role of *BCL-2* still controversial in cutaneous melanoma [31].

Due to the rarity of Head and Neck Mucosal Melanoma, and its etiopathogenesis still unclear, we evaluated the protein expression of C-MYC, *SOX10* and *BCL-2* nuclear markers, which have been associated to melanoma's metastatic risk.

MATERIALS AND METHOD

Tumor Specimens and Clinical Data

A total 10 cases of Primary Nasal Mucosal Melanoma from the State of São Paulo Cancer Institute (ICESP), Sao Paulo, Brazil and Department of Pathology, Medical School, University of Campinas, Brazil. Additionally, 19 cases of Melanomas (including head and neck primary mucosal melanomas, recurrent and metastasis) were organized in TMA from Department of Pathology, Yale School of Medicine, New Haven CT, USA. Clinical features, such as gender, age, site of tumor, and presence of metastasis; treatment; and outcomes were assessed. Five cutaneous melanomas were included as control cases. The histopathology of all cases was reexamined.

Immunohistochemistry Technique (IHC)

All Formalin-fixed paraffin-embedded (FFPE) sections were first baked and deparaffinised. Antigen

retrieval was accomplished by heating slides in Bond Epitope Retrieval Solution 1 for 30 minutes, followed by primary antibody incubation of C-MYC (clone Y69, dilution 1:100); *SOX10* (polyclonal, dilution 1:25) and *BCL-2* (clone 124, dilution 1:150) diluted with Dako Antibody Diluent (Ref S0809, DAKO) and the immunohistochemical staining were accomplished using labeled Leica Refine Red Detection System on Bond III Autostainer (Leica Biosystems, Buffalo Grove, Indiana). Finally, all sections were counterstained with CAT Hematoxylin (Biocar Medical) and stained slides were mounted with Cytoseal™60 (Ref 8310-4, Thermo Scientific).

Immunohistochemical Interpretation

Each case was classified and scored as: 0%; <5%; <10%; >10%; >25%; >50%; >75% and >90% according to the percentage of immunostained tumor cells. We only consider positive expression for C-MYC; *SOX10* and *BCL-2* proteins, when more than 25% of tumor cells were stained. All cases were analyzed by two researchers.

RESULTS

We observed 27/29 were positive to *BCL-2* and 28/29 cases showed *SOX10* expression in over 75% of tumor cells. However, we noticed variable nuclear expression for C-MYC in 15/29 of HNMM cases. *BCL-2* showed cytoplasmic pattern, on the other hand *SOX10* and C-MYC, both markers showing nuclear immunostaining (Figures 1 and 2).

DISCUSSION

In 2001, no significant difference of C-MYC expression was found between nevi and primary cutaneous melanomas, however 70% of all investigated melanoma metastases showed nuclear and cytoplasmic staining [18]. Human normal skin and nevi did not showed positive staining for C-MYC, however the expression of C-MYC was upregulated in primary melanoma and even more in metastatic melanomas [21]. In an immunocytochemical study, C-MYC oncoprotein was localized in the cytoplasm of tumor cells and it was expressed 14/30 cases of metastatic melanomas [32]. On the other hand, we found positive nuclear immunostaining of C-MYC in 51% of our HNMM cases: 7/16 primary tumor and 8/12 metastatic tumor.

The *SOX10* cytoplasmic and perinuclear expression was detected in 43% of primary melanomas with

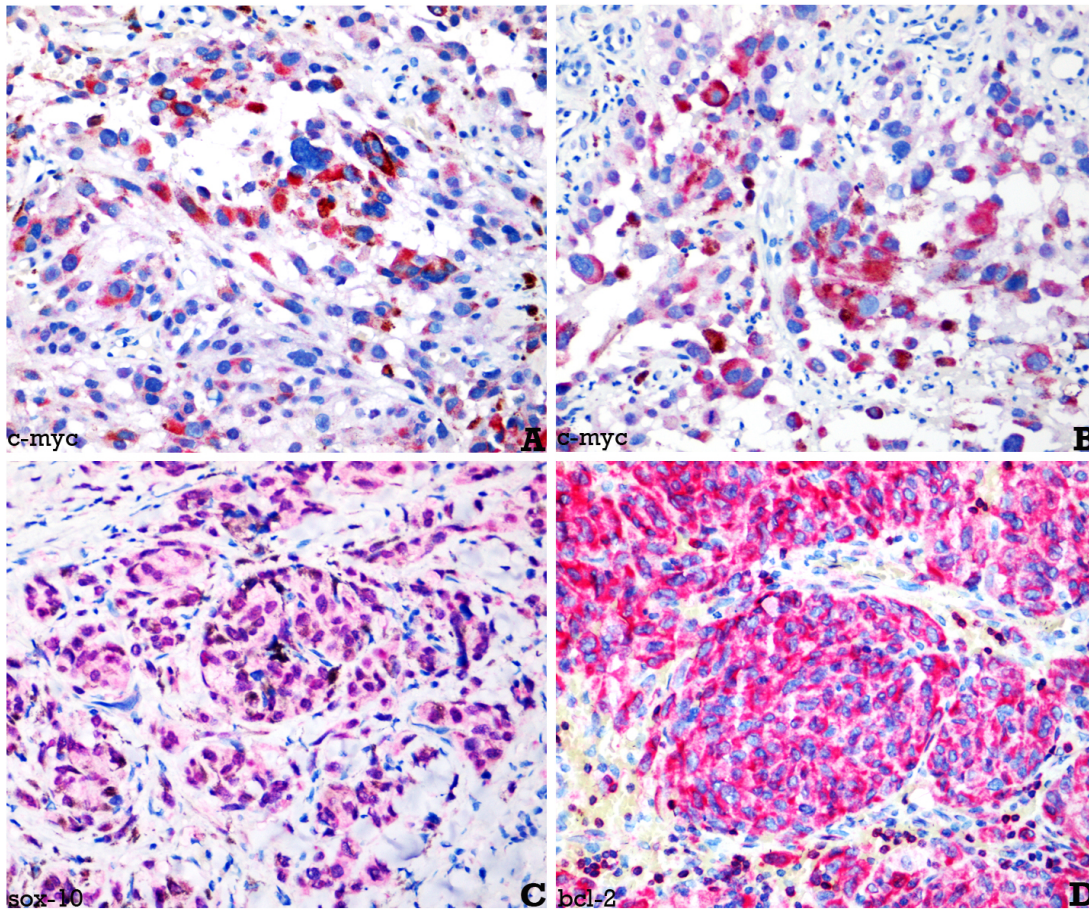


Figure 1: C-MYC, SOX-10 AND BCL-2 immunostaining by phosphatase alkalin technique (red chromogen).

A and B: immunostaining for C-MYC in primary HNMM, **C:** immunostaining for SOX-10 in primary HNMM, **D:** immunostaining for BCL-2 in primary HNMM (Magnification of 400X).

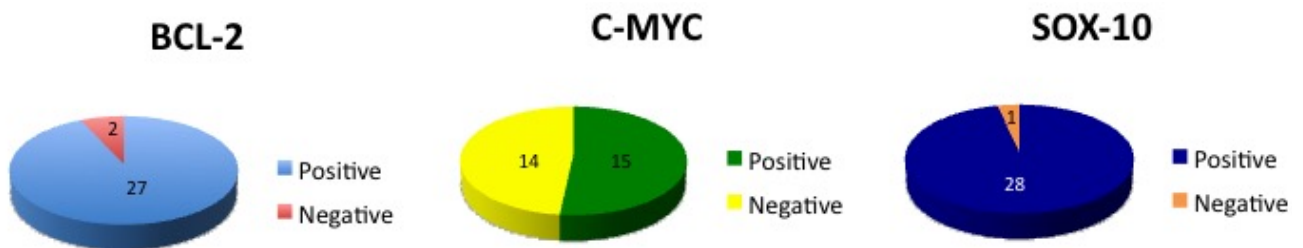


Figure 2: Total of positive and negative HNMM cases for BCL-2; C-MYC and SOX-10 protein analysis by immunohistochemistry.

varying staining intensity and percentages of positive cells, but its weak expression was positive in half of melanoma metastases cases, showing perinuclear and cytoplasmatic pattern [23]. In China, the nuclear expression of SOX10 in Sininasal Mucosal Melanoma was found in 100% of cases, and it is observed that the SOX10 staining pattern was stronger than S100 [26]. In 2014, a research group of MD Anderson Cancer Center found strong and diffuse positive nuclear staining in 100% of Fine-Needle Aspiration Smears from patients diagnosed with melanoma [33]. Not

different to the literature, we found positive nuclear immunostaining in almost all studied cases, except one single primary neoplasm case.

In a immunohistochemical study analysing the BCL-2 expression in human melanocytes and melanocytic tumors, they observed strong cytoplasmatic immunoreactivity inscattered melanocytes in the basal cell layer, and it was positive for all normal skin and primary cutaneous malignant melanomas, although it was positive or weak positive in 3/5 subcutaneous

melanoma and weak positive in 3/6 of lymph node metastasis [27]. In other IHC study, they found strong and/or BCL-2 expression in 18/23 in primary melanoma and 8/9 metastatic melanoma [28]. In a study of expression of apoptosis inhibitor in human melanoma, BCL-2 expression was positive in 14/15 superficial spreading and nodular malignant melanoma and 12/15 metastatic melanoma [34]. In 2007, a group in Australia evaluated different types of melanocytic lesions: compound nevus; dysplastic nevus; melanoma \leq 1.0mm; melanoma $>$ 1.0mm; subcutaneous metastases and lymph node metastases by immunohistochemic expression of BCL-2 was expressed in: 100%; 100%; 100%; 90%; 63%; 35% respectively. They observed that reduction in the BCL-2 expression was evident in thick melanoma and in tumor with high dermal mitotic rates, suggesting that it was related to events associated with disease progression [35]. Another Brazilian group, they evaluated BCL-2 expression three types of cutaneous melanoma metastases by chi-square test and it was observed: lymph node (74,3%); subcutaneous (85,7%) and visceral (82,3%) among 50 patients [36]. In a head and neck mucosal melanoma study in 64 patients was found 75%; 75% and 69% for BCL-2 positive expression in: initial, recurrent and metastatic tumors. They suggested that BCL-2 in initial mucosal melanomas predicts better prognosis [37]. All results found in the literature are controversial to our results of 93% of BCL-2 positive expression, because all metastatic tumor were expressed.

According to ours results, we suggest that BCL-2 and SOX10, should be good adjuvative biomarkers for HNMM, however lower expression of C-MYC has not shown to play main role in the HNMM development, thus further molecular biology studies should corroborate this present study.

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