Carcinoembryonic Antigen Cell Adhesion Molecule 1(Ceacam1) as Biomarker in Melanoma Management

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ABSTRACT

Currently, TNM classification is the only landmark for determining the prognostic and therapeutic management in melanoma. Variation in natural and therapeutic evolution of melanomas classified in the same TNM stage requires the development of a more complex classification system.

The international organizations involved in establishing protocols for melanoma treatment consider as mandatory the identification of new biomarkers for melanoma management. Biomarkers are in the center of the current research effort to improve tumor therapeutic strategies throughout early detection and diagnosis, precise staging, prognosis information, individualized mono or combined therapy, monitoring treatment response and recurrences.

Lately, new data emerged about carcinoembyonic antigen cell adhesion molecule 1 **(CEACAM1)** as a key molecule in several nodal intra and intercellular signaling pathways, with multiple functional and structural roles. CEACAM1 expression in melanoma is often described in the advancing edge of the tumor and has been associated with increased melanocytes invasion and migration. CEACAM1 is also expressed on B, T lymphocytes, natural killer cells and myeloid cells, modulating innate and adaptive immune responses.

This chapter will focus on the promising role of CEACAM1 in melanoma treatment, in monitoring melanoma patients, in assessing the response to immunotherapy and in completing the standard immunohistochemical panel used in melanoma examination.

Abbreviation: **anti-CTLA4** = Anti Cytotoxic T-lymphocyte–associated antigen 4; **anti–PD1**= Anti programmed death-1 protein; **anti–PDL1**= Programmed death ligand-1protein; **LDH** = Lactate dehydrogenase; **CEA** = Carcinoembryonic antigen; **AJCC** = American Joint Comittee on Cancer; **HMB45**= human melanoma black 45; **S100B** = S100 calcium-binding protein B; **MHC**= Major histocompatibility complex

INTRODUCTION

Biomarkers are in the center of the current research effort to improve melanoma management through out early detection and diagnosis, precise staging, prognosis information, individualized mono or combined therapy, monitoring treatment response and recurrences.

The latest achievements in understanding genomic variability between patients but also between primary tumor and corresponding metastases, molecular mechanism of melanomagenesis and the complexity of the host-tumor immune interactions reveal the heterogeneity of the disease and the need of new markers that may help to guide therapeutic decisions.

Melanoma biomarkers are tumour- or host-related factors, either tissue or serum based, and can be grouped in diagnostic biomarkers, prognostic biomarkers (estimate the risk to progress) and predictive biomarkers (predict the response to certain treatment) [1-3].

Several diagnostic biomarkers help to differentiate between benign nevi and malignant disease using immunohistochemistry (HMB-45, Tyrosinase, Microphthalmia-associated transcription factor **(MITF),** S-100, SM5-1 Chondroitin sulfate proteoglycan 4 **(CSPG4)**, p16, MUM-1, Mel-5, melanocortin-1, and PNL2), RT-qPCR assays and fluorescence in situ hybridization **(FISH)** analysis [4].

Conventional tissue prognostic markers include Breslow thickness, growth phase (radial vs. vertical), presence or absence of ulceration, Clark level, density and type of TILs, mitotic rate and sentinel lymph node involvement [3].

Various biomarkers are also evaluated through IHC, FISH, DNA microarrays, RT-PCR, comparative genomic hybridization **(CGH)**, microRNA expression profile to establish the

correlation between their level and melanoma staging, tumor progression, or survival: enzymes (Lactate dehydrogenase, Tyrosinase, Cyclooxygenase-2, Matrix metalloproteinases), soluble proteins or antigens (Vascular endothelial growth factor, Osteopontin, Galectin-3, heparin- and chitin- binding lectin YKL-40, Melanoma inhibitory activity, C-reactive protein, Cell adhesion molecules, Melanoma-associated antigens, MAGE, Melanoma antigen recognized by T cells 1, tumor-associated antigen 90 **(TA90)**), S100 proteins, melanin-related metabolites (5-S-cysteinyl-dopa, L-Dopa, 6-Hydroxy-5-methoxyindole-2-carboxylic acid) and circulating cell-free nucleic acids (miRNA-221, miRNA-29c) [5].

By contrast, there are few predictive markers: genetic tests for BRAF, c-Kit, MEK is used to predict response to certain inhibitors. To date there is no valid biomarker to predict the response to immune checkpoint inhibitors. [3]Circulating tumor cells **(CTCs)** were identified in 29% of patients with primary invasive melanoma and in 62.5% of metastatic melanoma patients [6-8]. Increased expression of melanoma cell adhesion molecule on CTCs correlated with a poor response to chemo-, radio- or IL-2 therapy [9].

All of the current markers have some disadvantages, but proteins that facilitate tissue invasion and metastasis (members of the CAM family and MMPs) are most likely associated with melanoma prognosis. Increased expression of these proteins is statistically significant associated with worse disease free survival or mortality outcomes [10].

Lately, new data emerged about carcinoembyonic antigen cell adhesion molecule 1 **(CEACAM1)** as a key molecule in several nodal intra and intercellular signaling pathways, with multiple functional and structural roles, as interference with cell-matrix adhesion and enhancing of cell motility through modulating of N-cadherin [11,12]. CEACAM 1 (also referred as C-CAM, biliary glycoprotein BGP, CD66a) is a complex glycoprotein linked to the cell membrane by a carboxy-terminal transmembrane anchor; it belongs to the carcinoembryonic antigen (CEA) family of the immunoglobulin superfamily. CEACAM1 expression in melanoma is often described in the advancing edge of the tumorand has been associated with increased melanocytes invasion and migration [13,14].

CEACAM1 is also expressed on B, T lymphocytes, natural killer cells and myeloid cells and through homophilic interactions modulates of innate and adaptive immune responses [15]. CEACAM1 inhibits natural killer cells activity, inhibits effector functions of tumor infiltrating lymphocytes (TILs), such as cytotoxicity and IFNγ release. Upregulation of CEACAM1 induced by IFNγ on melanoma cells that survive TIL-mediated attack renders them even more resistant [15-19].

CEACAM1 may lead to promising strategies in melanoma treatment, in monitoring melanoma patients, in assessing the response to immunotherapy and in completing the standard immunohistochemical panel used in melanoma examination.

MATERIALS AND METHODS

A literature search using the search engine "Pubmed" was conducted in March 2016 with the following keywords: melanoma, malignant melanoma, metastatic melanoma and CEACAM1and 46 articles were found. All papers were published after the year 2001.

RESULTS

Relevance of CEACAM1 Expression in Melanoma Management

Normal melanocytes donot express CEACAM1 [14]. CEACAM1 expression was studied on primary, metastatic melanoma, melanoma cell lines and tumor-infiltrating lymphocytes.

Thies and al showed that 40% of primary melanomas expressed CEACAM1 [13]. Studying superficial spreading melanoma (SSM), Gamblicher reported significantly higher CEACAM1 expression in thick SSM compared to thin SSM, dysplastic nevi and benign nevi. In SSM the intensity of the CEACAM staining increased with the depth of the tumor, positively correlating with Breslow tumor thickness and Clark level [13,20].

CEACAM1 is more frequently expressed in lymphocytes from melanoma patients and is constantly expressed on cells from tumor-infiltrating lymphocytes, exerting inhibitory effects [13,19]. CEACAM1 staining in vessels was not correlated with prognosis [13].

70% of CEACAM1 positive primary melanomas and only 10% of CEACAM1 negative primary melanomas metastasized in the cohort studied by Thies [13]. Almost 90% of the metastatic lesions from cutaneous melanoma express CEACAM1, as Ortenbergand his team reported [21]. The intensity of CEACAM 1 membranous positivity increased from primary tumors to lymph nodes and distant metastases in a small series of patients [22].

Regarding uveal melanoma, Katib noticed a similar distribution of CEACAM1 in primary and metastatic tumors [23].

The increase in tumor thickness is associated with an increased risk of regional lymph node involvement, raising from 2-5% in tumors thinner than 1mm to 34% in tumors thicker than 4mm [24]. The survival for patients with lymph node metastatic melanoma is dependent on lymph node tumor burden, with a40 % reduction in the 5-year survival that ranges from 78 % in stage IIIA, 59 % in stage IIIB to 40 % in stage IIIC [24]. In AJCC 7th edition of staging system for melanoma there is no lower limit of size to define nodal disease and micrometastases identified by immunohistochemistry are specifically included [25].

In clinically negative sentinel lymph nodes, to identify melanoma cells in current practice it is used immunohistochemical staining for S100, melanA and human melanoma black 45-HMB45 and the detection rate is improved with 10-20% compared to hematoxylin and eosin **(HE)** staining alone. Thies et al studied sensitivity and specificity of immunohistochemical markers for detection of melanoma cells in primary melanomas, sentinel lymph nodes and distant metastases

[26]. The authors evaluated the expression of cell adhesion molecules CEACAM1 and L1 compared to expression of standard markers MelanA, S100 and HMB45. The sensitivity of CEACAM1 and L1 markers was 87-93% in primary melanomas and 60-95% in their corresponding metastatic lymph nodes, similar with data from other studies [27]. CEACAM1 and L1 are also highly specific for melanoma cells, while MelanA, S100 and HMB45 are not, so they can be used in differentiating malignant cell from benign nevus cell inclusions in the capsule of sentinel lymph nodes that could otherwise lead to false positive diagnosis of stage III melanoma [26].

Two different antibodies for CEACAM1 were compared and the study highlighted the superior sensitivity of monoclonal antibody 4D1/C2 to the commercial NCL-CD66a. Antibodies 4D1/C2 against CEACAM1 have shown a higher specificity and sensitivity for melanoma cells in lymphatic and hematogenous metastases and the pathologists could add them to the standard list of antibodies [26-28].

CEACAM1 distribution strongly correlated with clinical evolution, suggesting that CEACAM1 expression could be and independent factor for the risk of metastasis with a better predictive value than Breslow index, regardless of the presence of ulceration or mitotic rate. For melanoma cases with similar stage according to American Joint Comittee on Cancer **(AJCC)** classification, CEACAM 1 status could optimize the current stratification, being a promising parameter for an ultrastaging[13].

TREATMENT WITH ANTIBODIES AGAINST CEACAM1

Being an immunogenic skin cancer, melanoma may benefit from active immunotherapy. Despite the current optimized targeted therapy (BRAF-oncogene inhibitors) or immune checkpoint inhibitors ((anti Cytotoxic T-lymphocyte–associated antigen 4 **(anti-CTLA4)**, anti programmed death-1 protein **(anti –PD1)**, programmed death ligand-1protein **(anti –PDL1)**), the treatment of metastatic melanoma is unsatisfactory. The last mentioned may induce severe immunologic side effects as they are not tumor specific. By contrast, CEACAM1 is a tumor specific antigen that may be an ideal target for antibody-based therapy, with limited risk for autoimmune adverse events [29].

CAM Biotherapeutics **(CM24)** and Agenus Inc developed two specific monoclonal antibodies for blocking CEACAM1 functions, reversing the inhibitory action on the NK and activated T cells and improve the antitumoral response of the endogenous immune system [30,31]. In 2018 will be available the results of a phase I trial (NCT02346955) taking place in 2 centers from the USA and Israel [32].

DISCUSSIONS

To date, for risk stratification TNM classification is the only landmark for determining the prognostic evaluation and therapeutic management in melanoma. Variation in natural and therapeutic evolution of melanomas classified in the same TNM stage requires the development of a more complex classification system.

The international organizations involved in establishing protocols for melanoma treatment consider as mandatory the identification of new biomarkers for melanoma management.

For the patients diagnosed with stage I disease, surgical resection by itself is usually considered curative, but the risk of recurrences ranges between 1%-12%, highlighting the heterogeneous behavior of melanoma and the urge to refine prognostic models to differentiate "low" and "high" risk patients in early stages [33]. CEACAM1 up regulation has been associated with increased melanoma cell invasion and migration and could be used to identify "high risk" melanoma cases [14].

Resting melanoma cells that can became active after variable periods of time are responsible for a recurrence risk of nearly 30 % instage II patients [34].

Lack of a general consensus regarding the follow up schedule for stage I and II resected melanomas determined Kluger and colleagues to propose a multiplex, plasma-based protein biomarker panel that included CEACAM, ICAM-1 (intercellular adhesion molecule 1), osteopontin, MIA (melanoma inhibitory activity), GDF-15 (growth differentiation factor 15), TIMP-1 (tissue inhibitor of metalloproteinase 1), and S100B to improve melanoma recurrences detection with a sensitivity of 74% and consecutively decrease the number of nonessential tests [35].

Increased sensitivity and specificity of CEACAM1 immunohistochemical staining refine detection of melanoma cells in sentinel lymph nodes and accurate recognition of stage III melanoma.

LDH is the only serum biomarker included in AJCC classification, correlates with tumor burden in stage IV disease, but it is unsatisfactory in evaluating progressive disease [36,37].

S100 calcium-binding protein B **(S100B)** plays a limited prognostic role in patients with stage I and II disease beyond initial histopathologic diagnosis, but it was found to be superior to LDH in revealing early distant metastasis [38,39]. Both markers indicate poor outcome, shorter disease-free and overall survival [40,41].

CEACAM1 is found at a low level in healthy persons, but in melanoma patients it is concordant with the tumor mass that secrets CEACAM1 and it significantly correlates with LDH level [42].

In metastatic melanoma serum levels of CEACAM1 also correlated with S100, disease activity and overall survival rates. In patients with active disease plasma level of CEACAM1 was inversely linked to time to death from melanoma. Most of the patients evaluated had normal levels of LDH, so CEACAM1 assessment proved to be useful for a more accurate prognostic prediction [43,44].

CONCLUSIONS

The high heterogeneity of melanoma raises the need to identify new biomarkers to define subsets of tumors with particular behavior in order to adjust management strategies.

In this chapter we illustrated expression profile of CEACAM1 in primary and metastatic melanoma and its roles in tumor aggressiveness and invasiveness. The wide distribution of CEACAM1 in metastatic melanoma qualifies it as an attractive target for immunotherapy, alone or in combination. CEACAM1 could be a useful biomarker in all melanoma stages, for diagnosis (tisular and serological assessment) and prognosis (optimizing the current classification system and providing information for ultrastaging).

Due to both its promising potential and scarce literature, additional robust studies are needed to establish CEACAM1 role in melanoma management.

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