Immunohistochemistry (IHC) is an excellent technique for labeling detection of selected cell proteins, which exploits the principle of antigen-antibody specific binding in biological tissues [1]. First implemented by Albert Coons in 1941 [2], over time it had achieved great success for diagnostic purposes (diagnostic IHC), then for prognostic ones (prognostic IHC) [3]. Several molecular pathways are altered in skin melanoma and non-melanoma skin cancers and some of these can be targeted in oncotherapy [1]. Therefore, IHC application has increased and can predict those tumors which are likely to respond to targeted cancer therapy (predictive IHC), by detecting the presence or high expression levels of altered gene products.

**Diagnostic IHC**

Among the most used diagnostic markers for melanoma cells, there are: anti-S100, anti-MelanA, anti-MITF (microphthalmia transcription factor), anti-Sox10 (sry-related hmg-box 10), anti-Melanosome and melanoma cocktail. Quite similar to calmodulin, S100 protein is a low-molecular weight protein characterized by two calcium-binding sites in a “helix-loop-helix” conformation [4]. It in fact plays crucial roles in calcium homeostasis, cytoskeleton dynamics, protein phosphorylation, cell growth, cell differentiation and inflammatory response [5]. It is soluble in 100% ammonium sulphate at neutral pH, hence its acronym [4]. S100 protein is not specific for the melanocyte lineage, being present in all cells derived from the neural crest [6]. MelanA protein, also known as MART1 (melanoma antigen recognized by T cells 1), is a transmembrane protein made up by 118 amino acids; its fragment consisting of nine amino acids (27–35) is bound by MHC (major histocompatibility complex) class I molecules, which present it to cytotoxic T-lymphocytes on the cell surface [7]. The MelanA antigen is more specific for the melanocyte lineage than S100, and its diagnostic utility resides in staining melanoma cells in a homogeneous manner from the epidermis downward, allowing easy recognition of lentiginous or pagetoid diffusion and sub-epidermal foci of microinvasion [1]. MelanA expression is regulated by MITF, which is a basic “helix-loop-helix” leucine zipper transcription factor involved in lineage-specific regulation pathways of many cell types, melanocytes included [8]. In humans, mutations of MITF can lead to melanoma, Waardenburg syndrome and Tietz syndrome [9]. MITF IHC is sensitive but not specific since it can be expressed also by osteoclasts and mast cells [10]. In melanocytic cells, there is evidence that MITF may also regulate the SOX10 gene expression and, therefore, SOX10 IHC has been recently introduced for diagnostic purposes [11]. Melanosomes are cell organelles deputy to synthesis and storage of melanin in normal melanocytes and melanoma...
cells, from which melanin can be released to adjacent keratinocytes [12]. Hmb45 (human melanoma black 45) is the monoclonal antibody which reacts precisely against melanosomes [13], its staining pattern is usually zonal/shallow in nevus and diffuse/deep in melanoma, making differential diagnosis easier [13]. Inside melanosomes, the melanin production is catalyzed by the rate-limiting oxidative enzyme tyrosinase – for this reason, the anti-Tyrosinase monoclonal antibody is one of the melanoma cocktail components, together with Mart1 and Hmb45 [14].

**Prognostic IHC**

IHC has a limited value in determining the prognosis of skin melanoma, which is in fact related to microstaging attributes, such as Breslow’s depth, Clark’s level, ulceration, regression, mitotic hot-spot, growth phase, lymphovascular invasion and tumor-infiltrating lymphocytes [15–23]. The current AJCC (American Joint Committee on Cancer) staging system uses Breslow’s depth and ulceration as main prognostic determinants [24–26]. In this context, IHC can facilitate a better definition of the abovementioned histopathological attributes; for example, juxtaposing the labeling index of Ki67 proliferative antigen to the mitotic count [1], or disclosing the type of growth phase by evaluating the expression of the anti-apoptotic molecule cyclin D1 [1,27–30], or identifying the subtypes and rates of lymphocytes around and inside the tumor, with particular reference to CD4–CD8+ T-killer lymphocytes [31–34], or highlighting the hematic and lymphatic endothelia of the capillaries involved by neoplastic spread [35]. To reach this last goal, anti-Pecam1 (platelet endothelial cell adhesion molecule 1) or anti-Podoplanin antibodies can be used, resp. [36]. Among the tumor suppressor genes, CDKN2A encodes for p16ink4a protein, which plays an important role in cell cycle regulation by inhibiting cell cycle progression from G1 to S phase [37]. The CDKN2A gene is frequently altered in malignant melanoma; therefore, IHC for the mutant form of p16ink4a can be used in the diagnosis of microinvasive melanoma, in order to distinguish it from in situ melanoma or dysplastic nevus, both characterized by a lower immunolabeling and an indolent course [1].

**Predictive IHC**

Today, the main genes involved in melanoma genesis susceptible to predictive IHC investigation are – BRAF, NRAS, CDK4, KIT [38]. Mutations in BRAF and NRAS are responsible for 50% and 20% of all melanomas [39]. Vemurafenib and dabrafenib are V600 mutant B-Raf inhibitors approved for the treatment of late-stage melanoma [40]. The
growth signal transduction triggered by V600 mutant B-Raf can be also mitigated inhibiting MEK (MAPK/ERK kinase) proteins, members of MAPK (mitogen-activated protein kinase) signaling cascade, thus favoring cell cycle block and apoptosis [40]. The most known MEK inhibitors used in the treatment of metastatic melanoma are trametinib and cobimetinib [41]. Binimetinib, another MEK inhibitor, has been tested in a randomized phase III clinical trial for N-Ras Q61 mutant melanoma, becoming the first molecularly targeted therapy for NRAS mutant patients [42]. Palbociclib and ribociclib are CdK4 inhibitors that are under study for melanoma and other drug resistant tumors, such as estrogen receptor positive breast cancer [43,44]. Imatinib and nilotinib are Kit inhibitors available if necessary; the former is active when the mutation occurs in exon 11 of KIT, while the latter when it occurs in exon 17 [45]. More recently, a lot of attention has been paid to melanoma drug resistant tumors, such as estrogen receptor positive breast cancer [43,44]. Imatinib and nilotinib are Kit inhibitors available if necessary; the former is active when the mutation occurs in exon 11 of KIT, while the latter when it occurs in exon 17 [45]. More recently, a lot of attention has been paid to melanoma immunotherapy, it is a type of passive immunotherapy aimed to enhance preexisting anti-tumor responses of the organism [46]. In this regard, two molecules, Cta-4 and PD1, have attracted interest of many researchers [47]. Cta-4 is a surface receptor expressed by activated T-lymphocytes, able to transmit an inhibitory signal of self-tolerance to T-lymphocytes, functioning as an immune checkpoint for normal and neoplastic cells [48]. Ipilimumab is the monoclonal antibody developed to stop the above-mentioned inhibitory signal by binding to Cta-4. This event is preliminary to melanoma cell destruction by cytotoxic T-lymphocytes [49]. PD1 is a surface receptor of activated T-lymphocytes, which plays an important role in down-regulation of the immune system and promoting self-tolerance [50]. Its ligand, known by the acronym PD-L1 (programmed death-ligand 1), is highly expressed in 40–50% of melanoma and, hence, the role of PD1 in melanoma immune evasion is now well established [51]. Nivolumab and pembrolizumab are anti-PD1 human monoclonal immunoglobulin G4 capable to block the interaction between PD1 and PD-L1 (immune checkpoint blockade), favoring the melanoma cell attack by T-cells [52]. In 2017, the Food and Drug Administration has approved the use of pembrolizumab also for unresectable or metastatic solid tumors with mismatch repair deficiency or microsatellite instability [53]. Strong evidence showed that microsatellite instability is a frequent condition in malignant melanoma, as well [54]. In line with what has been recently hypothesized by other authors [55,56], my working group has noticed, in daily clinical practice, that the best therapeutic results of pembrolizumab occur in those patients affected by melanomas with mismatch repair deficiency (Fig. 1). Therefore, before choosing the most suitable treatment, the biopsy specimen should be also tested for Mih1, Msh2, Msh6 and Pms1 [57], the well-known DNA mismatch repair proteins in humans.

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References


