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Serum Markers in Clinical Management of Malignant Melanoma

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1. Introduction

The incidence of cutaneous malignant melanoma (CMM) is increasing in the Western world, despite the implementation of prevention campaigns for several years. Early detection, targeted at-risk populations helps to identify patients with beginning melanoma lesions, but despite these efforts, many patients - often young,- present with thicker tumors (with a higher Breslow, over 1mm - Breslow is a measurement in mm of the vertical thickness of the primary tumor-). [1]

A late diagnosis is also often associated with a greater Breslow thickness index and a greater risk for invasion of regional lymph nodes (stage III), or distant metastatic lesions (stage IV) [2]. CMM usually progresses from an in situ proliferation in a radial growth pattern. Then appears the vertical growth phase which is a major event for the dissemination of cells, as it allows cells to migrate deeper into the dermis, the lymphatic vessels and blood flow.

In the seventh revision of the American Joint Committee on Cancer (AJCC) staging for melanoma (2009), patients can be divided into four stages: stage I and II disease (local) stage III (locoregional disease) and stage IV (metastatic disease). In this ranking, the only serum marker that was built for clinical use is the lactate dehydrogenase (LDH) as the serum LDH was confirmed in multivariate analysis to be an independent predictor even after taking into account site and number of metastases [2]. The value of LDH is still often discussed in local/locoregional conditions.

Surgery is the mainstay of treatment of melanoma. The major concern after diagnostic excision of the primary lesion is whether CMM has already metastasized or not. Indeed many arguments emphasize that early detection of melanoma metastases may improve the prognosis of
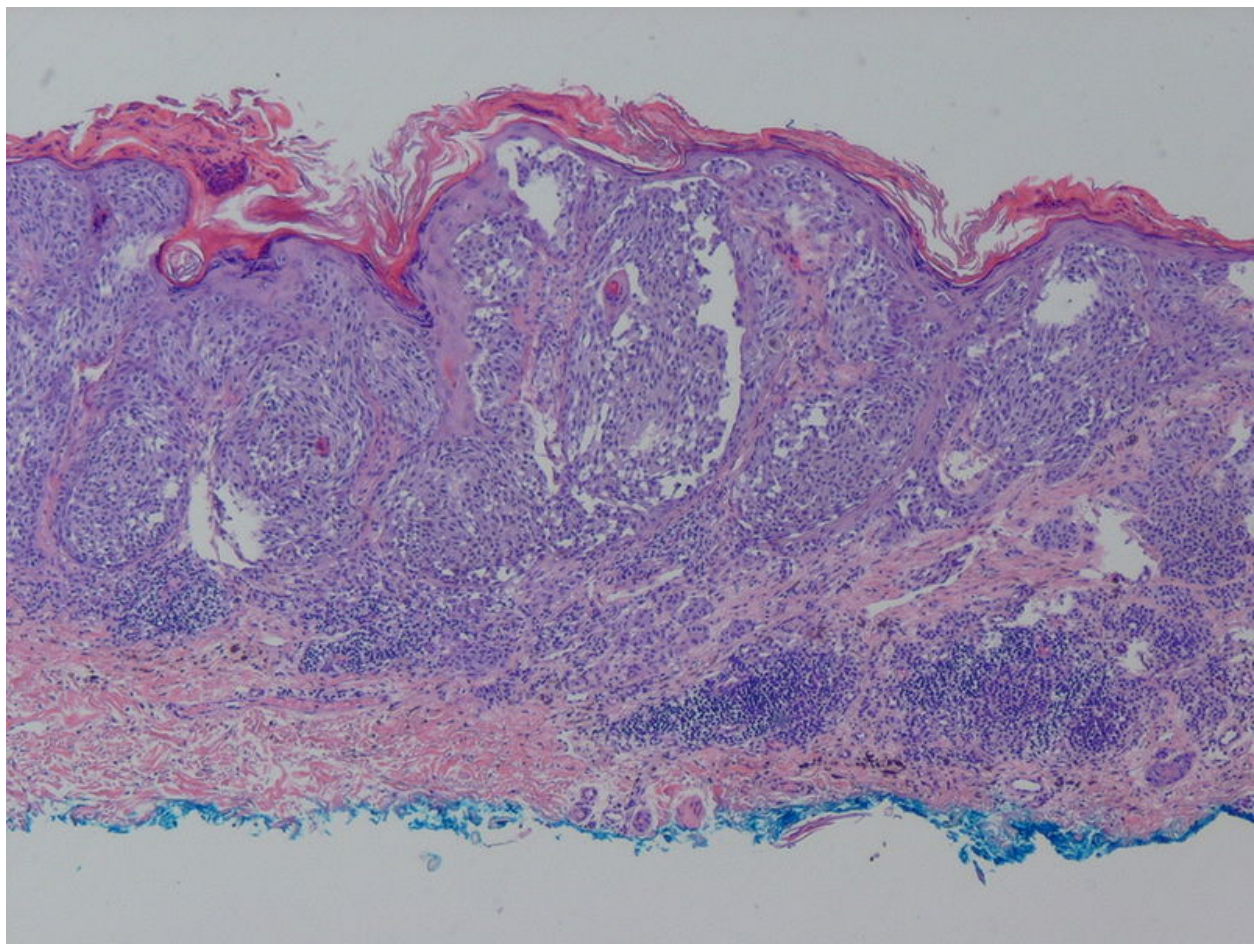
Figure 1. Breslow index measures in mm the thickness of the lesion

patients, at least for some of them. To date, no marker for early detection of melanoma metastases is unanimously recognized.

A melanoma patient around high risk (high risk of recurrence) can be defined as a patient with a 50% risk of relapse within up to 10 years, despite optimal initial surgical treatment. These high-risk patients should be carefully monitored and treated if possible with adjuvant therapeutic strategies. Interferon-α and, more recently, ipilimumab have been proposed as adjuvant therapies, but their effect on survival is still a matter of debate. To date, no predictive marker of response has been described.

The metastatic process involves the spread of cancer cells in locoregional or distant anatomic sites via the lymphatics and / or blood flow. In the case of melanoma, circulating cells can find a suitable microenvironment in the sentinel node (the first lymph drainage lymph node area), other lymph nodes or distant organs (lymph nodes, liver, lungs, brain, bone).

In fact, the understanding of the biology and mechanism of metastatic cascade provides new molecular targets and can help us to discover new biomarkers. Biomarkers can be divided into diagnostic markers for disease detection and prognostic and predictive markers, which should predict response to treatment. Cancer biomarkers consist of many molecular structures such

as proteins, peptides, DNA, mRNA. Interest is the fact that these markers can be found in the tissues, cells and / or body fluids. Also viable melanoma cells can also be found in the peripheral blood of melanoma patients. We limit ourselves in this article to the description of serum molecular markers in CMM.

The ideal biomarker should be a molecule easily detectable in the serum of a patient who presents a growing tumor. The biomarker should have a sufficient sensitivity and specificity to minimize false negatives and false positives. Sensitivity refers to the proportion of patients with confirmed disease who have a positive test for a biomarker, whereas specificity can be defined as the proportion of healthy individuals with a negative test. Previous studies have shown that many molecules that may be involved in oncogenesis and cancer spread can be found in the serum of cancer patients in particular patients with melanoma, but their sensitivity and / or specificity are still debatable. These molecules can be produced and secreted or excreted into the bloodstream directly by melanoma cells or indirectly by destruction of melanoma cells by chemotherapy, immunotherapy or combination therapy [3].

Below, we detail the most important molecules in serum that have been described as a biomarker for CMM.

2. Main serum markers in CMM

2.1. Lactate Dehydrogenase (LDH)

As mentioned above, this enzyme has been considered as the main serum prognostic parameter in patients with metastatic melanoma (AJCC stages III and IV). Numerous studies have validated LDH as the factor most predictive of patient outcome, and this independently and statistically significant. This led to a stratification of the AJCC :patients with metastatic melanoma with high levels of LDH are designated as M1c whatever the site of metastasis [2].

Note, however, that Hamberg stated that in a series of 53 patients with stage IV AJCC melanoma only 38% had elevated LDH, suggesting that elevated LDH is not the ideal marker for this condition [4]. Moreover, in a multivariate analysis of 64 patients with AJCC stage IV melanoma Hauschild has failed to demonstrate the independent prognostic value of LDH [5]. It should be recalled that the LDH assay can be falsely positive due to hemolysis and other factors, including hepatitis.

However, Weide et al also insist in a study of 855 patients on the prognostic value of LDH [6].

2.2. C-Reactive Protein (CRP)

CRP is a nonspecific inflammatory parameter that may have a role in the detection of melanoma progression. This protein is produced by hepatocytes as acute phase response of nonspecific inflammatory processes.

Elevated serum CRP was associated with a poor prognosis in various cancers. Deichmann et al. analyzed the prognostic significance of CRP compared to the LDH patients AJCC stage IV
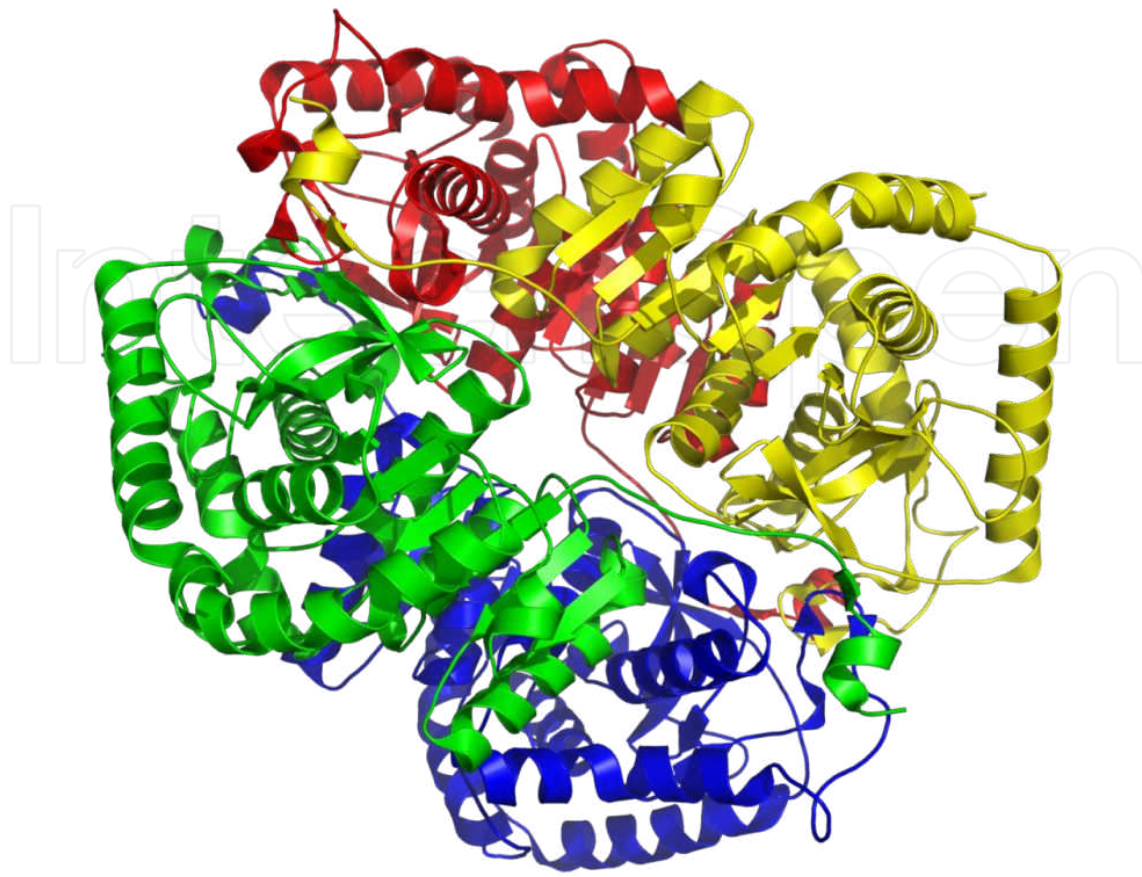
Figure 2. LDH is a tetrameric enzymle and consists of several sub-units M and H, encoded by two different genes (Chromosome 11 and 12)

melanoma. With a definition of a threshold 3mg/dL, the identification of a stage IV can be done with a sensitivity of 76.9% and a specificity of 90.4%. In another prospective study of 67 patients, Deichmann found that CRP was the only prognostic factor even reliable [7-9]. These results are debated.

2.3. S100-β proteins (S100B)

Serum S100B is described as more related to the tumor burden and thus reflects both the clinical stage and tumor progression (the higher the rate of serum S100B, the greater the tumor burden). It may therefore be used to monitor the effectiveness of therapy whatever the type of treatment (surgical, chemotherapy, immunotherapy). Retsas et al. have suggested the use of S100B instead of LDH in the classification system of the AJCC while other authors consider that S100B has no added value when comparing the sensitivity and specificity of the CRP and LDH [10]. For some, S100B has probably become the most useful marker in clinical practice, but it interest seems to be limited to advanced stages III and IV [11]. In stages I and II S100B provides no independent prognostic information.
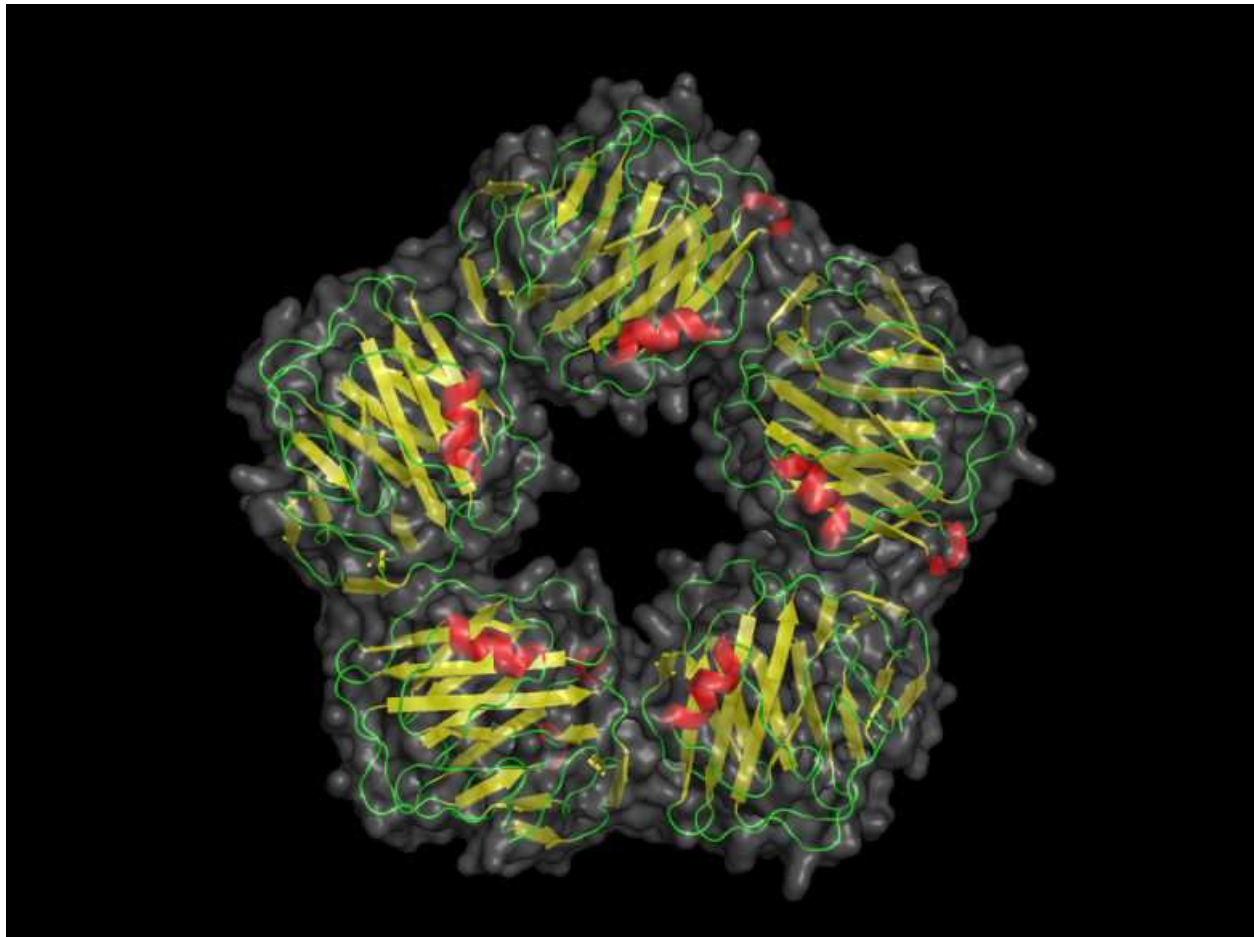
Figure 3. Molecular structure of CRP, five sub-units each comprising 206 amino acids. The gene responsible for its synthesis is located on chromosome 1.

Moreover, it must be remembered that S100B is not specific to melanoma and its serum levels may be elevated in healthy subjects, patients with cancer of non-melanoma skin, neurological disorders, tumors of the nervous system central, and even in various gastrointestinal cancers, and patients infected with HIV.

2.4. Melanoma Inhibiting Activity (MIA)

The roles of this protein are multiple among them modulation of cell growth and cell adhesion. MIA rates are higher in the group of patients relapsing after initial surgery. Some authors consider that the sensitivity of the two molecules S100B and MIA is equal. For other authors, MIA is superior to LDH and CRP. In children and pregnant women (after week 38), MIA is increased and serum levels should be avoided in these two groups [12].

2.5. Galectin-3

Gal-3 has been described to be overexpressed in malignant melanocytic lesions and its concentration in the serum of patients with melanoma is increased by the joint action of
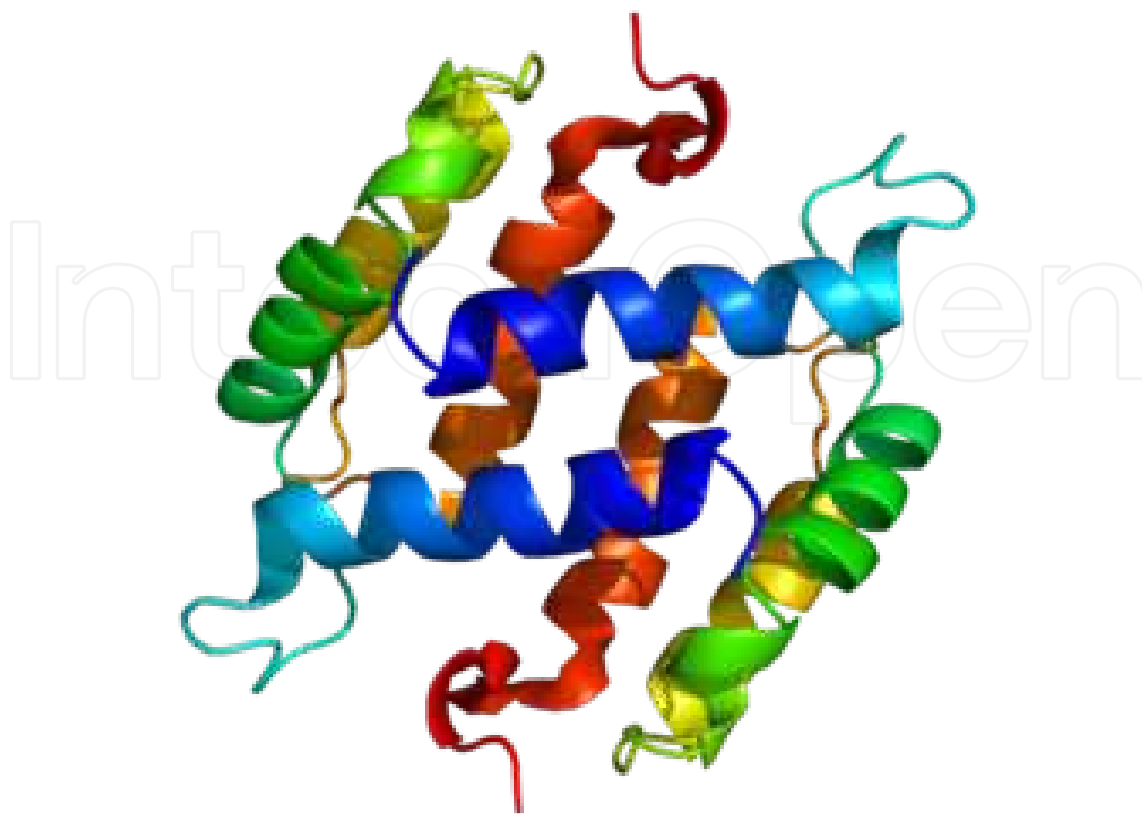
Figure 4. S100B is a 21-kD dimeric protein, consists of two β subunits. This protein is a member of a family of 19 protein and was first isolated from bovine brain in the mid sixties. S100B is expressed by glial cells and melanocytes and is produced by brain tumors and melanomas. The roles of S100B are probably multiple and underestimated.

melanoma cells and inflammatory cells. Gal-3 plays an important role in cell proliferation, cell differentiation, cell adhesion, cell migration, angiogenesis and metastasis. Thus, Gal-3 deserves special attention. Clarification of the role of extracellular Gal-3 should help us to understand the significance of elevated serum levels of this molecule in patients with advanced melanoma [13].

3. Other molecules and molecular approaches

3.1. Melanoma Associated Antigens (MAA)

Malignant transformation of melanocytes is associated to changes in gene definition. This leads to the expression of melanoma associated antigens molecules called (MAA), which are more or less specifically associated with the malignant phenotype (Table 1). In fact sometimes these MAA can also be expressed in normal melanocytes. MAA play an important role in triggering the immune response agains melanoma cells. These antigens were mainly identified by immunological approaches, including in vitro and in vivo reactions and serological tests. These antigens can be defined by their ability to interact with T cells or B and peptides derived from
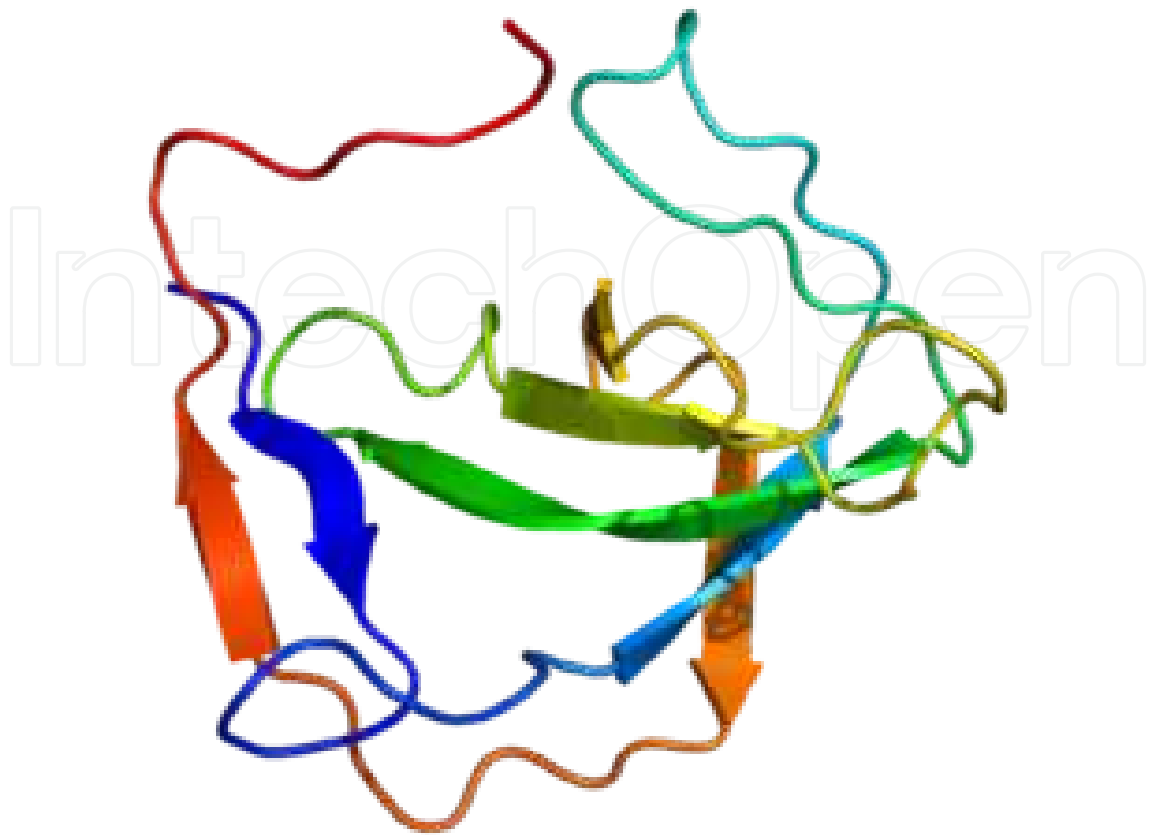
Figure 5. MIA is a 12kDa soluble protein, whose role has been characterized as an inhibitor of cell autocrine growth. It can be expressed by melanoma cells and chondrocytes.

these antigens have been used to induce or maintain a specific immune response. Mage-1 was the first identified MAA and now belongs to a large family of at least 12 antigens differentially expressed by benign and malignant melanocytic cells. Immune responses to these genes can be used as markers of progression and / or immunological response.

Tyrosinase RT-PCR detection in patients with melanoma is correlated with a higher risk of relapse (55% of these patients have a clinical relapse), but the specificity of this technique has yet to be optimized [15, 16]. When combined with a dosage of S-100, Domingo-Domenech showed that tyrosinase RT-PCR adds valuable prognostic information in patients with S-100 <0.15µg / l, although the team showed that S-100 had a higher predictive value. Curry et al. suggested that RT-PCR detection of tyrosinase can be useful to determine a subgroup of patients with an increased risk of metastases [17].

Profiling of autoantibodies associated with certain MAA was different by Sabel et al as potentially useful to select patients with melanoma who should benefit from the research of a sentinel node.

These results have yet to be validated [18].
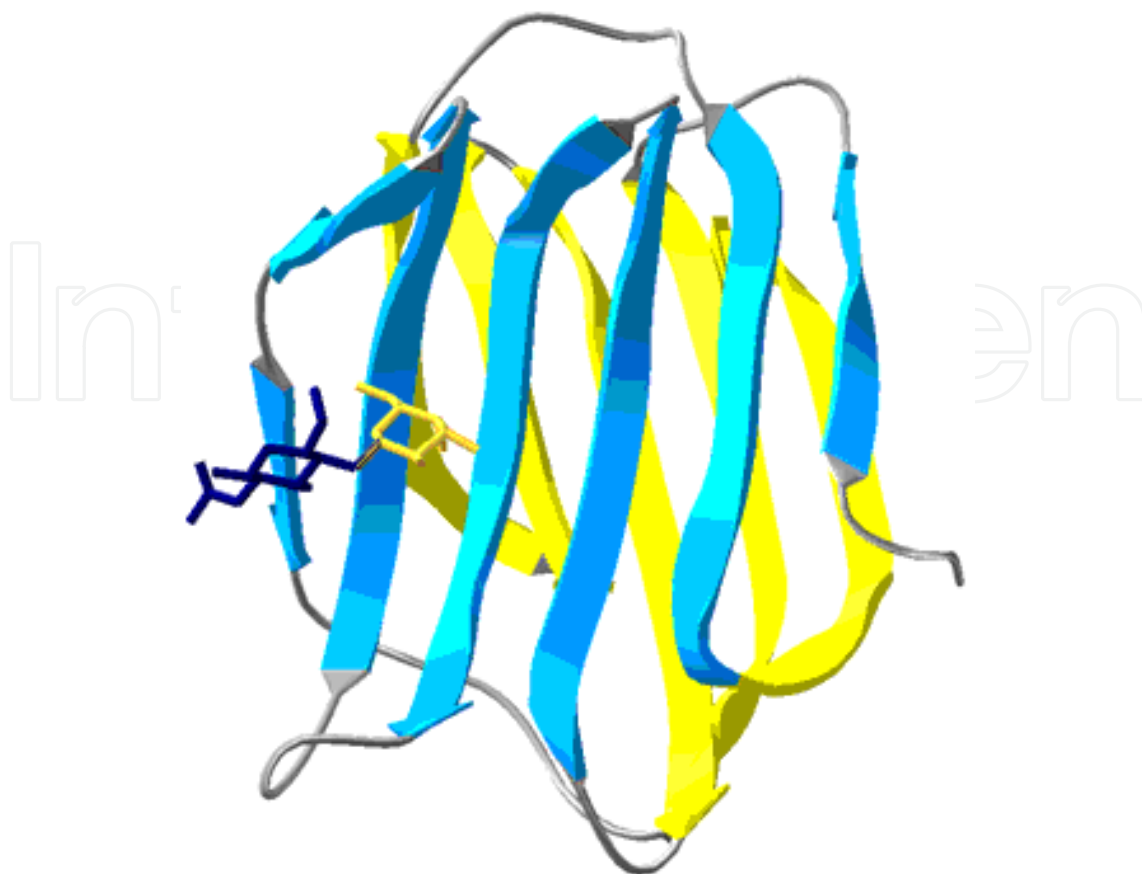
Figure 6. Galectin-3 is a member of the family of lectins that can bind to β-galactoside residues. Many members of the galectin family are differentially expressed in cancer. Gal-3 is a molecule that contains an NH2-terminal domain, a COOH-terminal domain.

3.2. Melanine metabolites

5-S-cysteinyl dopa (5SCD) is a precursor of phaeomelanin and is produced by melanocytes and melanoma cells, as a product of the binding of a molecule named dopaquinone and highly reactive with cysteine. 5SCD is detectable in the urine and serum of melanoma patients and correlates with disease progression. In patients with progressive disease, the level of 5SCD can rise before the onset of clinical signs. A comparative report stated that with the LDH and S100B, 5SCD has an interesting value even if the authors of this report concluded that S100B could be considered as the most sensitive of the three markers. Due to the effect of UV exposure on melanin, the use of this 5SCD as a biomarker may be limited in Caucasians, whereas its use in other populations, particularly in Japan, would be more reliable. In addition, patients with metastatic but non-pigmented melanoma lesions do not usually have elevated serum 5SCD. 3,4-dihydroxyphenylalanine (L-DOPA) is the first metabolite involved in melanogenesis and its plasma levels were also correlated with melanoma progression and tumor burden, as well as the ratio of plasma L-dopa / L tyrosine-which represents an index of the activity of tyrosinase and tyrosine hydroxylase. Stoitchkov et al. showed that the latter ratio has predictive value, especially in patients with stage III, and advocated the use of multiple biomarkers [19].

Antigen	HLA restriction
Oncospermatogonal antigens	
MAGE-A1	A*01, A*03, A*24, A*28, B*3701, B*53, Cw*0201, Cw*0301, Cw*1601
MAGE-A2	A*0201, B*3701
MAGE-A3	A*01, A*02, A*2402, B*3701, B*44, DR*11
MAGE-4	A*0201
MAGE-A6	A*3402, B*3701
MAGE-A10	A*0201
MAGE-A12	A*0201
MAGE-B1	A*0201
MAGE-B2	A*0201
BAGE	Cw*1601
GAGE-1	Cw*6
LAGE-1	A*0201
PRAME	A*24
NY-ESO-1	A*02, A*31
DAM-6	A*02
Melanocytic differentiation antigens	
Tyrosinase	A*01, A*0201, A*2402, B*44, DRβ1*0401
MART-1/Melan-A	A*0201, A*02, B*4501
Gp100	A*0201, A*03, A*0301, A*1101, A*2402, C*0802, DRβ1*0401
TRP-1	A*31
TRP-2	A*31, A*33, A*0201, C*0802, A*68011
MC1R	A*0201
Mutated antigens	
MUM-1	B*44
CDK4	A*02
B-catenin	A*24
P15	A*24
GnT-V	A*02
TPI	DRβ1*0101
Annexin II	DRβ1*0401
CDC27	DRβ1*0401
Oncogene-derived antigens	
HER2/Neu	A*0201

Table 1. Melanoma associated antigens (adapté de Visser et al. [14]).

3.3. Matrix Metalloproteinases (MMP)

MMPs are a family of 24 structurally related endopeptidases. These zinc-dependent enzymes are defined by their own substrates and can lyse components of the extracellular matrix (ECM) (eg, collagen type IV, which is a major component of the basement membrane by gelatinases such as MMP-2 and MMP-9) and play a role in angiogenesis and the renewal of the ECM. MMPs can also cleave different molecules such as other proteinases, proteinase inhibitors, growth factors, adhesion molecules and thereby modulate the inflammatory response, the process of tumor growth, tumor invasion and metastasis.

A balance between MMPs and tissue inhibitors of metalloproteinases (TIMP) can be broken by upregulation of MMP and TIMP downregulation, this is shown in malignant phenotype.

Another important role, angiogenesis, has been attributed to MMP, which could allow a therapeutic target possible. Batimastat (BB-94, a synthetic broad-spectrum inhibitor of metalloproteinases), for example, has shown efficacy for inhibiting angiogenesis of liver metastases in a mouse model.

MMP overexpression has been reported in melanoma progression, and elevated serum MMP, ie MMP-1 and MMP-3, have been correlated with poor survival [20].

3.4. Cytokins, chimiokins and their réceptors [21]

Chemokines are small polypeptides signaling can bind to and activate G protein-coupled receptors, a family of seven transmembrane molecules. Multiple roles have been attributed to these chemokines, and are involved in tumor transformation and metastatic process. The differential expression of these chemokines and their receptors may explain the organ specificity of metastases.

Melanoma cells expressing the chemokine CXCL8, also known as interleukin-8 (IL-8) have been described and a report showed that serum levels of IL-8 are associated with tumor burden and a poor prognosis. This track is interesting and could be exploited for therapeutic purposes. In vivo studies have indeed shown that anti-IL8 humanized antibodies are able to reduce tumor growth and angiogenesis.

A recent study of 29 serum cytokines assayed simultaneously in 179 melanoma patients (versus 378 control individuals, healthy) showed a profile of specific serum cytokines in patients compared to controls: higher serum concentrations of interleukin (IL)-1alpha, IL-1beta, IL-6, IL-8, IL-12p40, IL-13, G-CSF, MCP-1, MIP-1alpha, MIP-1 beta, IFN-alpha, TNF-alpha, EGF, VEGF and TNF receptor II. [22, 23, 24].

3.5. Growth and angiogenic factors

Angiogenesis is an important step in tumor growth as it ensures the supply of oxygen and substrates to tumor cells. This process is in fact the result of complex interactions between pro-angiogenic factors and anti-angiogenic released by tumor cells, endothelial, epithelial, mesothelial cells and leukocytes. Vascular Endothelial Growth Factor (VEGF) has been

described as a potent mitogen for endothelial cells and its expression, which can be increased in hypoxic conditions, was also correlated with tumor progression and poor prognosis.

Different VEGF have been described, but none has been reported as an independent prognostic factor. At most a few studies have correlated the presence / absence of certain molecules in very specific situations, eg serum VEGF-C is decreased in patients with metastatic cutaneous metastases [25].

An imbalance in the ratio of serum angiopoietin 1 and angiopoietin 2 than could, according et al Gardizi sign a metastatic progression. [26]

3.6. Cell surface and adhesion molecules

3.6.1. Integrins

Integrins are cell components that ensure adhesion to other cells, ECM, or other proteins. Other important roles are played by integrins such as the transmission of information between the extra-and intracellular space, and angiogenesis.

Integrins are heterodimeric receptors consisting of two subunits α and β,. On the basis of their common subunit, heterodimers can be classified αv, $\beta 1$ $\beta 2$ integrins. The main integrins involved in the progression of melanoma include av$\beta 3$ (vitronectin receptor and fibronectin), $\alpha 2\beta 1$ (collagen), $\alpha 4\beta 1$ (fibronectin) and $\alpha 6\beta 1$ (laminin).

Some reports have shown that increased serum concentrations of β integrins were associated with shorter survival. The clinical impact of this has not yet been defined.

3.6.2. CD44

CD44 is a transmembrane glycoprotein surface, originally described as a lymphocyte receptor. CD44 is a cell surface receptor for hyaluronic acid, While some studies have highlighted its role in tumor invasion and metastasis, it is clear that no study has identified a prognostic value for serum CD44.

3.6.3. ICAM-1

ICAM-1 is a new intercellular adhesion molecule, located in the cell membranes of leukocytes and endothelial cells. ICAM-1 is a ligand of LFA-1 (lymphocyte function-associated antigen-1) in T cells, B cells, macrophages, and neutrophils. Leukocyte migration is facilitated by the binding ICAM-1/LFA-1. One study showed that serum ICAM-1 is increased in patients with metastatic but has no independent prognostic value in multivariate analysis [27].

3.7. Others

Ever, new publications emerge with new molecular perspectives (protein profiling, micro-RNAs dosage,...).

Luo et al showed in well constructed paper that isoenzymes of aldehyde dehydrogenase 1A (ALDH) appear as stem cell markers of melanoma, but their presence in serum and their prognostic significance has yet to be defined [28].

Adhesion molecule type 1 related to carcinoembryonic antigen (CEACAM 1) has also recently been shown as a promising biomarker by an Israeli team: measuring the serum in a retrospective study is correlated with metastatic progression and survival patients with melanoma [29].

The utility of serum DNA of mutated BRAF gene has also recently been presented as a prognostic factor and predictor of response to biochetherapies. This would probably be very useful for patients treated with vemurafenib. These results need to be confirmed [30].

4. Discussion

Cancer is a major cause of morbidity and mortality in our society. It shows a huge price and has many devastating effects. Melanoma is a tragic example of these realities.

The prognosis of melanoma is closely linked to early diagnosis. Current treatments have limited effectiveness, and surgery remains the mainstay of treatment. Better treatments are certainly necessary, even with the arrival of molecules such as ipilimumab or vemurafenib that enable new perspectives for our patients with metastatic disease.

In the past, the only prognostic factor in melanoma patients has been limited to histology (tumor thickness) and the location of the primary tumor. These parameters are important, but were supplemented by numerous clinical variables, pathological and biological, particularly in patients with advanced melanoma. Recently, the use of serum markers, alone or in combination, has been proposed to refine the prognosis of a patient in order to ensure proper tracking and predicting the potential benefits of therapy. More specific or nonspecific markers of melanoma can be measured in the serum of patients, and in most cases, these markers are directly correlated with the tumor mass.

Among these biomarkers, LDH and S100B serum biomarkers appear with an independent prognostic value, although disputed by some. In advanced melanoma, their dosage is probably more accurate and sensitive than CRP levels (LDH and CRP are obviously more accessible and measured) as shown by some studies, but not ideal. Even if the LDH is incorporated into the new AJCC classification, for some authors, S100B is superior in terms of prognostic value [31].

To a lesser extent due to poor sensitivity or lower specificity, CRP, MIA, and Gal-3 can also be considered as interesting biomarkers. The dosages of new other molecules should be included in future prospective clinical protocols, distinguishing their prognostic value (patient outcome) and predictive value (response to treatment).

Storage conditions of serum should also be made clear in all the articles as they have a major influence on results and therefore conclusions. This suggests that a standard methodology should be set in order to compare the published studies [32].

Research into new biomarkers in melanoma is important issue because i twill lead to better understand the biology of this tumor, and thus it improve patient monitoring, early detection and treatment of secondary lesions and open new perspectives for targeted therapies. Multiple molecular changes of melanoma progression are currently intensively studied.

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