chapter 7

Concluding remarks and summary

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Different types of melanocytic lesions such as common acquired, dysplastic, congenital and other nevi, are recognized by their histologic appearances. In this respect, to distinguish harmless nevi from those with increased risk for malignant transformation still remains difficult for some of the melanocytic nevi. Different complex mechanisms that control the stages of differentiation and the transformation of normal epidermal melanocytes give rise to these various types of melanocytic lesions. It has been postulated that these lesions occur due to subtle changes in normal physiological processes such as proliferation, up or downregulation of critical genes, mutations, and the *de novo* cell-cell or cell-ECM interactions. The primary goal of this thesis is to evaluate markers by their topographic antigen distribution and gene expression profile to distinguish common acquired nevi from dysplastic ones and also to discriminate primary melanoma with minimum risk to those with increased potential for metastasis.

The introduction of this thesis (**chapter 1**) outlines a brief overview on the current knowledge about the biology of melanocytes and the development of different types of melanocytic tumors from benign to metastatic lesions. In addition, several markers on the proteomic and RNA level studied by immunohistochemical and molecular approaches to better understand the biology of melanoma is also provided. Against the background of such overview, it is apparent that despite the availability of many markers, no single molecular and pathological criterion can as yet unequivocally distinguish the different stages of melanocytic neoplasia. Consequently, the study design of this thesis was set up to analyze early changes in phenotypes of normal melanocytes, transformation from nevi into melanoma, and to gain insight into the molecular pathology of melanocyte neoplasia. Such study design resulted into five experimental chapters, each of which describe data that are regarded essential and may

improve diagnosis of melanocytic lesions with the risk of developing into malignant forms, and eventually will lead to a better understanding of the tumor biology of melanoma.

With a panel of monoclonal antibodies (MoAbs), antigenic profiles have been studied retrospectively by immunohistochemical analysis (**chapter 2**). The MoAbs used in this study recognize differentiation (gp-100, PAA, TRP-1) and progression (HLA-DR, MAA-1, MAA-2) related antigens. The immunohistochemical analysis of different melanocyte related antigens described in this study, suggests that micro-anatomy related heterogeneity of antigen expression, rather than just a score of positive versus negative, may be of value in the discrimination of common acquired nevi from dysplastic nevi. Based on these results further prospective studies on the primary melanomas and secondary spread from such lesions will be valuable for understanding the role of tissue micro-environment in malignant transformation and metastasis and melanocytic cells.

In continuation to the previous results, antigenic profiles in frozen tissue sections of primary melanomas and their metastases within the same patient have been studied with the panel of MoAbs that was also used in chapter 2. In this way recognition of tumor progression stages based on the evaluation of antigenic profiles should be feasible (**chapter 3**). As a result, expression of differentiation markers (PAA, TRP-1) was observed in the radial growth phase of the tumor, whilst a decreased or loss of expression was found in the vertical growth phase of the melanoma lesion. In contrast, the antigenic profile of the melanoma associated antigens (MAA-1, MAA-2) was either positive or negative during melanoma tumor progression, of which the positive melanoma cells showed a strong membranous immunoreactivity with the surrounding stromal cells. This study showed that by comparing antigenic profiles of primary melanoma-metastasis pairs of the same patient, consistent differences in topographical antigen expression were obtained. Consequently, this indicates that differences in antigenic profile may result in consistent diagnosis of melanocytic lesions.

In conclusion, differences in the topographic antigen distribution and the intensity of the staining reflect the different stages of melanocyte transformation and melanoma tumor progression. One should therefore, advocate the use of a panel of markers known to be important in melanocyte biology in conjunction with the histopathological criteria to improve diagnosis of different types of benign or malignant melanocytic lesions.

Although identification of different stages of melanocytic lesions by immunnohistochemical means is an attractive method, it still remains difficult to understand completely why some melanocytes transform and eventually develop into malignant melanoma. In order to better understand this process we need additional markers to comprehend this complex phenomenon. One approach (**chapter 4**) is to investigate the gene expression levels, which eventually will assist in the identification of more candidate markers, particularly in the early stages of melanocyte transformation by a combination of subtractive hybridization and differential display (SHD). Based on the RNA expression profiles, two novel (pCMa1, pCMn2) and the known genes (Rab5b, U1A, β2 tubulin, TI-227H), are potential markers. The differential expression patterns of Rab5b and two novel gene fragments (pCMa1, pCMn2) were further assessed in melanocytic cells in this study. pCMa1 was expressed more in metastatic melanoma as compared to other melanoma cells. In contrast, the expression of pCMn2 was evident both in non-metastatic and metastatic melanoma cells but not detectable either in normal melanocytes or nevus cells. The Ras-related protein Rab5b showed lower levels of expression in highly metastatic melanoma cells as compared to other melanocytic cells. To our knowledge no cDNA fragments have been documented in literature using SHD that identified markers with such distinct expression profiles in early and late stages of melanocytic transformation.

Although the identified cDNAs represent potential molecular markers, evaluation of the gene expression profiles in cultured cells as compared to native tumor samples, and the

molecular and/or functional relevance is needed to establish the actual value of these markers in melanocyte transformation and tumor progression of melanoma. In this respect we explored the molecular characteristics of pCMa1 and compared its *in situ* profile in different melanocytic lesions (**chapter 5**). Two major transcripts of approximately 0.45 and 4.0 kilobase (kb) were identified by Northern blot analysis. The absence of a polyadenylated signal sequence and an extensive open reading frame in the cDNA of the 0.45 kb transcript, suggests that pCMa1 does not encode a protein. Moreover, the detection of multiple, complementary transcripts of variable sizes, and the genomic localization of pCMa1 to chromosome 11p15.1-2 indicates that its expression is regulated in a complex manner. Furthermore, RNA *in situ* hybridization analysis in different melanocytic lesions of various stages also revealed a characteristic expression profile for the 4.0 kb transcript of the novel marker pCMa1. pCMa1 was not detectable in normal skin melanocytes, but was transiently expressed in melanocytic nevi. This transcript was also expressed in primary melanoma lesions, but was not detectable in melanoma metastases. Therefore, unraveling the regulation of gene expression by a more thorough analysis of genomic sequences and genes surrounding pCMa1 could be very interesting to resolve the nature of the longer transcripts. Although, the function of the corresponding gene of pCMa1 has not been established in this study, the chromosomal localization, gene expression and *in situ* profile are suggestive for a role in melanocyte transformation.

Despite the identification of several candidate markers, the information is still incomplete to understand thoroughly the mechanisms involved in melanocyte transformation and melanoma tumor progression. Further search for additional markers is needed and can be accomplished by studying altered expression of genes in other diseases. In this effort a candidate gene *GOA* (*gene overexpressed in astrocytoma*) was identified by serial analysis of gene expression (SAGE) which can distinguish normal brain from astrocytoma brain tumor

cells (**chapter 6[a]**). The cDNA of *GOA* is 2286 bp long, contains a poly-A tail and a consensus Kozak sequence, encodes a 639 amino acid protein, and was found to represent a new member of the Ring finger B-Box Coiled-Coil (RBCC) proteins. This protein was overexpressed in glioma as compared to normal brain. Since it is known that melanocytes and glial cells are derived embryologically from the neural ectoderm, glioma and melanoma cells may share common genes, similar genetic changes, and biological properties that may reflect the neuroectodermal origin of the tumor cells. Furthermore, because malignant melanoma also has an affinity to metastasize to the brain, we extended the study as described in chapter 6[a] and evaluated the *in situ* expression profile of *GOA* in a variety of melanocytic lesions of different stages (**chapter 6[b]**). *GOA* was not detectable in normal skin melanocytes, but was expressed in melanocytic nevi. This may suggest a role for *GOA* in the development of melanocytic nevi. *GOA* was also expressed in cultured melanoma cell lines of different metastatic capacity. Evaluation of the gene expression profile in cultured melanocytic cells as compared to the native tumor samples may suggest an additional role for *GOA* in the tumor progression of melanoma. This statement is further supported because *GOA* was mapped to 17q24-25, a chromosomal region that frequently displays gain or amplifications of genes in a number of other tumor types including melanoma.

Future perspectives to identify candidate markers in melanoma research can simply be accomplished by screening the public available databases. The resulting sequence information of the identified known and novel genes has become huge in public databases in particular due to the near completion of the human genome sequencing project. However, the challenge will be to put the wealth of information in perspective and to fully understand tumor progression and metastasis by relating differential expression of genes with phenotypic and functional differences in the cell type studied. Unfortunately, the newly identified markers are routinely not used in conjunction with the existing histological and clinical parameters in

diagnostic pathology of melanoma. Therefore, the possible diagnostic, predictive and/or prognostic value by using a set of markers known to be important in melanocyte biology should be advocated. In addition the gene expression profile established for each marker will eventually result in a better understanding of early phenotypic and malignant transformation of melanocytic cells. Perhaps in the near future, the identified markers will improve therapeutic modalities in the management of patients diagnosed with malignant melanoma.