Omics in melanoma – what have we learnt and what is the potential impact for patient management?

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The objectives of high thoughput technologies in cutaneous melanoma are, as in other solid tumours:

(1) To characterise key events and molecular pathways during tumour progression, meaning for melanocytic tumours to characterize key defects associated with nevus/primary melanoma and primary melanoma/metastasis transition,
(2) and thus to discover new therapeutic targets.
(3) To study if there is a prognostic transcriptional signature.

In some solid tumours, another objective of genomic studies is to identify a predictive signature for therapeutic response that would allow to better focus aggressive therapies and to enrich clinical trials. But for melanoma, the absence of any demonstrated active systemic therapy makes this objective of limited impact.

For years, most academic pathologists handling melanocytic tumours have considered that the whole tissue coming from small melanocytic tumours should be fixed for conventional diagnosis and that it would not be ethical to freeze any part. In the same time, some others have considered that given the lack of any benefit provided by any medical intervention apart from excision at any stage of the disease, it would not be ethical to seriously hamper research in destroying tumour RNA. In order to gather a sufficient number of cases of frozen primary melanomas, we decided in 2003 to create an international consortium to share different resources: an annotated retrospective biobank of frozen primary melanomas with appropriate informed consents, an annotated retrospective multicentric biobank of fixed primary melanomas with long follow-up (median: 8 years), an omics platform with high-density oligo-chips for transciptome and CGH array, a bioinformatics platform and a collaborative network (the EORTC Melanoma group) for biomarkers development and targets validation.

From the expression study, a group of 254 prognostic genes was identified. The top 60 genes represented a validated and reproducible signature prognosticating metastases or death. These 60 genes are mainly associated with replication or DNA repair. For replication, two pathways are over-represented: the replication origins firing genes (ROF) and the separation of sister-chromatids by securin. The ROF system is a fine tuning of helicases activity which role is to control that replication forks will divide only once despite the asynchronous replication along the DNA matrix. If the ROF pathway is impaired, the cell may enter into aneuploidy and chromosomal instability. Poor prognostic melanomas are characterised by a global overexpression of ROF-related genes; four out of ten of these MCM (Mini Chromosome Maintenance) genes, i.e. MCM-2, -3, -4, and -6 and geminin are in the prognostic signature. MCM-4 and MCM-6 immunoexpression is strongly correlated with metastasis free survival and OS. This prognostic value is maintained when age, sex, location of the primary tumour, thickness and ulceration are introduced in the multivariate model. The whole ROF system is locked by geminin that complexes CDT1 and CDC6. When CDT1 and CDC6 are released, they can recruit MCMs at the replication origins. When this interaction is altered, for instance when BRCA1-IRIS relieves geminin-CDC6 interaction, the helicases cascade becomes overactive leading to replication increase. The identification of this key pathway in melanoma progression opens new therapeutic perspectives and some molecules are being developed acting at the MCMs and geminin level.

hPTTG gene, coding for securin, is among the top genes of the prognostic signature. Securin has three known activities: it blocks the sister-chromatids separation in stabilising separase, it stimulates angiogenesis and it decreases *p53* transcription. Securin acts as an oncogene and provides a positive growth advan-

tage as it downregulates sister-chromatids separation and therefore avoids the cells entering into aneuploidy. P53 transcription inhibition leads to a decrease in p53-mediated apoptosis. Securin immunohistochemical expression is observed in vertical growth phase whereas melanomas in radial growth phase do not express securin.

Among 254 prognostic genes associated with primary cutaneous melanoma, 48 genes are DNA-repair genes therefore leading to an over-representation of this functional cluster. Overexpression of DNA-repair genes is associated with metastases or death. Increase in DNA repair capacity could explain spontaneous resistance of most melanomas toward radiotherapy and alkylating agents. In this 48 genes list, Nucleotide Excision Repair family genes, involved in xeroderma pigmentosum, and Base Excision Repair family genes are not represented. On the contrary, most of the DNA repair genes present in this group are involved in post-replicative repair of DNA lesions. This is in accordance with the hypothesis that aggressive melanomas need a fast and effective replication, and need to repair mistakes induced during replication. One of the genes of which overexpression is most evidently associated with poor prognosis is *topoisomerase 2A*. *Top2A* codes for an enzyme that is essential for replication and chromosomal segregation. Actually *Top2A* expression seems to be a consequence rather than a cause of cell proliferation. However, cells that overexpress *Top2A,* and PCNA with which it interacts, are much more resistant toward alkylating agents. Chemo-sensitisers interacting with TOP2A are being studied.

In parallel with expression genomics studies, CGH array studies have provided important information to refine the melanoma classification. The expression signature associated with *B-Raf* mutations reveals a strong association between *B-Raf* mutation and *CD63* overexpression ($P = 10^{-14}$). These results and their consequences will be discussed during the presentation.

To result in the development of a melanoma, normal melanocytes or nevus cells need to enter into genetic instability associated with amplifications, deletions or mutations. However, genomic studies results show that a metastasising melanoma needs to stabilise its genome. They strongly suggest that an increase in the replication ability is an early characteristic of primary melanoma and is associated with its metastasising potential.

In conclusion, a melanoma that will metastasise is a DNA replication machinery. Maximal efficiency and minimal mistakes are necessary to overcome replicative stress and telomeres maintenance. Post-replicative DNA repair genes expression is increased in these melanomas explaining their extreme resistance toward radiotherapy and alkylating agents. Mitotic activity, that is a strong and widely recognised independent prognostic factor, is the phenotypic reflect of a dysregulation in two major pathways that control replication: the replication origins firing and sister-chromatids separation. Stabilisation of the geminin/CDT1 and geminin/CDC6 interactions might be reinforced by drugs. *B-Raf* mutation impacts genes with serin threonin kinase activity as *CD63*. How these results and aCGH data impact melanoma classification will be discussed.

Conflict of interest statement

None declared.

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