Karolinska Institutet

Institution för onkologi-pathologi

MOLECULAR STUDIES IN DIFFERENT TYPES OF MELANOMA TUMORS - CORRELATIONS TO CLINICAL DATA

AKADEMISKA AVHANDLING
Som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Öron-Näsa-Hals föreläsningssalen, Karolinska Universitetssjukhuset, Solna

Tisdagen den 3 december, 2013, kl 13.00

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Stockholm 2013

ABSTRACT

Approximately 90% of melanomas arise from skin sites (known as cutaneous malignant melanoma; CMM), whereas the non-cutaneous melanoma (mucosal and ocular melanomas) are rare, accounting for about 10%. Familial melanoma accounts for up to 10% of patients diagnosed with CMM. Both genetics (e.g. *CDKN2A* and *CDK4* germline mutations, as well as polymorphisms in *MC1R* and other genes) and environmental factors (ultraviolet radiation) contribute to the induction of melanoma. The MAPK and the PI3K are the two most commonly activated signaling cascades in melanomas. Activation of these two pathways occurs frequently through alterations in *BRAF*, *NRAS* and *KIT* oncogenes. The involvement of these oncogenes in common CMM subtypes is well-studied. However, the frequency of mutations in *BRAF*, *NRAS* and *KIT* and also *PTEN* has not been well-characterized in the other rare melanoma subtypes, at least not in Caucasian populations. The overall aim of this thesis was to better define the molecular genetic alterations of *BRAF*, *NRAS* and *KIT* in different subtypes of melanomas and to correlate the mutation status with the histopathological features of the tumors and with the clinical parameters of the patients.

For the first project, Formalin-fixed paraffin embedded samples of primary familial and sporadic CMMs were collected from eight centers in Europe and Australia. The overall aim was to better define the frequencies of *BRAF* and *NRAS* mutations in familial melanoma with and without germline *CDKN2A* mutations. Overall, 89 tumors from patients with germline *CDKN2A* mutations, 46 from patients without germline *CDKN2A* mutations, and 50 sporadic melanomas were analyzed for *BRAF* exon 15 and *NRAS* (exon 2) mutations using direct DNA sequencing. The tumors were also evaluated for pERK and pAkt expression by immunohistochemistry (IHC). The *BRAF* and *NRAS* mutation frequencies detected in familial melanomas were 43% and 11%, respectively. These frequencies did not differ significantly between tumors from germline *CDKN2A* mutation carriers and non-carriers. The frequency of *BRAF* mutation (41%) and *NRAS* mutation (12%) in the sporadic melanomas did not differ significantly from that identified in the familial melanomas. Expression of pERK and pAkt was observed in 65% and 46% of the familial melanomas, respectively. Similar frequencies of pERK and pAkt expressions were observed in the sporadic melanomas.

In the second project, we analyzed a large number of a rare subtype of melanoma; sinonasal mucosal melanoma. In total, 56 primary tumors were screened for mutations in *KIT* (exons 11, 13 and 17), *NRAS* (exons 1 and 2) and *BRAF* exon 15 using direct sequencing. Twelve of the 56 (21%) tumors contained one mutation in these oncogenes; 2 tumors harbored *KIT* mutations, another 2 harbored *BRAF* mutations and 8 had *NRAS* mutations. The mutations were more frequently detected in tumors originated from the paranasal sinuses than from the nasal cavity (p=0.045). Patients with melanoma in the paranasal sinuses had a worse overall survival than patients with melanoma in the nasal cavity(p=0.027).

In the third project, primary and metastatic acral lentiginous melanomas were investigated for mutations in *BRAF* (exons 11 and 15), *NRAS* (exons 1 and 2), *KIT* (exons 9, 11, 13, 17 and 18) and *PTEN* (exons 1, 3-6 and 10-12) by direct sequencing. The data showed an identical mutation frequency of 15% (13 out of 88) of both *KIT* and *NRAS*, whereas *BRAF* mutations were found in 17% (15 out of 88) of the primary tumors. Of the 25 cases evaluated for *PTEN* mutations, only one tumor contained a mutation (4%). The *BRAF*, *NRAS* and *KIT* mutation status in 16 metastases was similar to that identified in the matched primaries. In comparison with *BRAF* wild-type tumors, *BRAF* mutated tumors were more commonly diagnosed in young individuals (p=0.028) and significantly associated with tumor location on the feet (p=0.039) and female gender (p=0.039). The anatomical site was an independent prognostic factor with better overall survival for patients with tumors on hand or subungual areas than those with tumors on the feet or under toenails (p=0.025).

In the fourth project, we evaluated 124 primary and 76 metastatic (including 73 matched metastases) CMMs for $BRAF^{V600E}$ expression by IHC using VE1 antibody. Overall, 55% (110 out of 200) tumors displayed a positive homogenous staining. There was a consistency in $BRAF^{V600E}$ staining between the matched primaries and metastatic CMMs. In 28 tumors a discrepancy was observed between the VE1 staining and the mutation analysis methods. Re-analysis of 25 tumors of the discrepant cases by pyrosequencing revealed a new $BRAF^{V600E}$ mutation in three cases, supporting the results seen with VE1 staining. In the remaining 22 tumors the results of the pyrosequencing and the initial mutation methods were similar. Overall sensitivity and specificity with VE1 antibody staining were 97% and 80%, respectively.

ISBN 978-91-7549-371-8