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Poster Communication Abstract – 6B.06

CDKN2A/P16^{INK4A} 5'UTR VARIANTS IN MELANOMA PREDISPOSITION: LOST IN TRANSLATION, SOMEWHERE

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The *CDKN2A* gene is the most common high penetrance susceptibility gene identified to date in melanoma families. While functional tests for determining the pathogenicity of missense germline mutations in the *CDKN2A* coding region have been developed, rare polymorphisms or sequence variants at the *CDKN2A/ p16^{INK4a}* 5'UTR, encountered during routine screening, are usually defined as variants with unknown significance after determining their frequency in control population and the cosegregation analysis in the family, when possible. We recently developed reporter assays to study a panel of *p16^{INK4a}* 5'UTR variants identified as heterozygous changes in patients from a hospital-based series of melanoma cases (c.-21C>T; c.-25C>T&c.-180G>A; c.-56G>T; c.-67G>C). Monocistronic as well as bicistronic luciferase-based reporter vectors were developed and used to test wild type and variant p16^{INK4a} 5'UTR activity upon transient transfection in melanoma-derived cells (WM266-4, G361 and SK-Mel-5) and in the breast cancer-derived MCF7 cells. Results revealed that the c.-21C>T variant had a strong negative impact on the reporter activity, similar to that of the known melanoma-predisposing mutation c.-34G>T, included as a control. The variants at -56 and at -25&-180 exhibited a milder impact, while results with c.-67G>C were dependent on the type of reporter vector. Quantification of the luciferase mRNA conducted in parallel with the luciferase assay indicated that the impact of the variants was mainly post-transcriptional. We also applied a polysomal profiling technique to measure allelic imbalance starting from heterozygous patient-derived cell lines and found that the c.-21C>T variant but also c.-56T>G and c.-67G>C exhibited lower association with the polysomes suggestive of reduced mRNA translation efficiency. A panel of eleven additional germline variants in the 5'UTR of *p16^{INK4a}* is being investigated. In particular we are focusing on the functional interactions between wild type and variant *p16^{INK4a}* 5'- and 3'-UTR sequences and on the impact the variants can have on

the targeting of the $p16^{INK4a}$ mRNA by microRNAs or RNA binding proteins.