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FUNCTIONAL IN VIVO SCRENNING OF NOVEL ONCOGENIC ENHACERS/SUPPRESSORS IN ZEBRAFISH MELANOMA MODEL

A model for melanoma progression has been developed in zebrafish based on most frequently mutated human gene in melanoma: BRAF. In a zebrafish melanoma model over-expression of histone methylase SETDB1 accelerates the onset of melanoma development. Most recently, Busch et al demonstrated that loss of chromatin modifier Kdm2aa causes BRAFV600E independent spontaneous melanoma in zebrafish. These observations are in line with the RNA analysis done previously in our lab on 15 congenital naevi versus 13 primary melanomas revealing potentially interesting genes whose functional consequences are only partially known. To functionally test candidate genes for the ability to accelerate melanoma transgenic zebrafish that over express candidate genes such as PIK3CA^{H1047R}, YAP-1, STK11, NRAS^{Q61K} and BRAF^{V600E} on a Tp53 mutant background was observed. The target genes were tested under 2 different promoters-MITF and SOX10. In addition to this, epigenetic genes MAPK14 and TET2 were tested for melanoma enhancer/suppressor in zebrafish melanoma of BRAF and NRAS mutant origin. Multisite gateway technology in combination with HiFi DNA assembly was used to create the expression constructs which were injected in 1 cell stage Nacre mutant embryos to create stable transgenic lines. The tumor incidence rate in Tg(mitf:BRAFV600E)p53(lf)mitf(lf) was 21. 4% compared to 100% in Tg(mitf:NRASQ61K)p53(lf)mitf(lf). The Tg(mitf:STK11)p53(lf)mitf(lf) did not show any phenotypic effect yet. The Tg(SOX10:BRAFV600E)p53(lf)mitf(lf) have a poor survival rate develop spontaneous tumors in the head region and Tg(SOX10:NRASQ61K)p53(lf)mitf(lf) are embryonic lethal. Further, the effect of MAPK14 andTET2 in melanoma progression were tested in Tg(mitf:BRAFV600E/NRASQ61K)p53(lf)mitf(lf) by over-expressing them in melanocytes. There has been no tumor formation in the transgenic line where MAPK14 was over expressed on melanocytes alongside NRASQ61K compared to the line where only NRASQ61K is over expressed in melanocytes resulting in tumor development within 5 weeks of birth. Furthermore, the differences in tumors of BRAF and NRAs mutant origin is being investigated through histological examination and RNA sequencing. Through the creation and analysis of several transgenic animals; one or more genes capable of accelerating/rescuing melanoma could be identified.

SPECIAL TECHNIQUES AND EQUIPMENT

Crispr-Cas-9, Gateway cloning, zebrafish handling, RNA sequencing