Droplet Digital™ PCR Success Story

Cancer Mutation Analysis

Validation of Single Nucleotide Polymorphism Array Analysis with Droplet Digital PCR in Uveal Melanoma Reveals Potential for Clinical Application

Pieter van der Velden

Ophthalmology Department
Leiden University Medical Center
Leiden, The Netherlands

"Our laboratory research focus is preclinical analysis in uveal melanoma."

Research Background

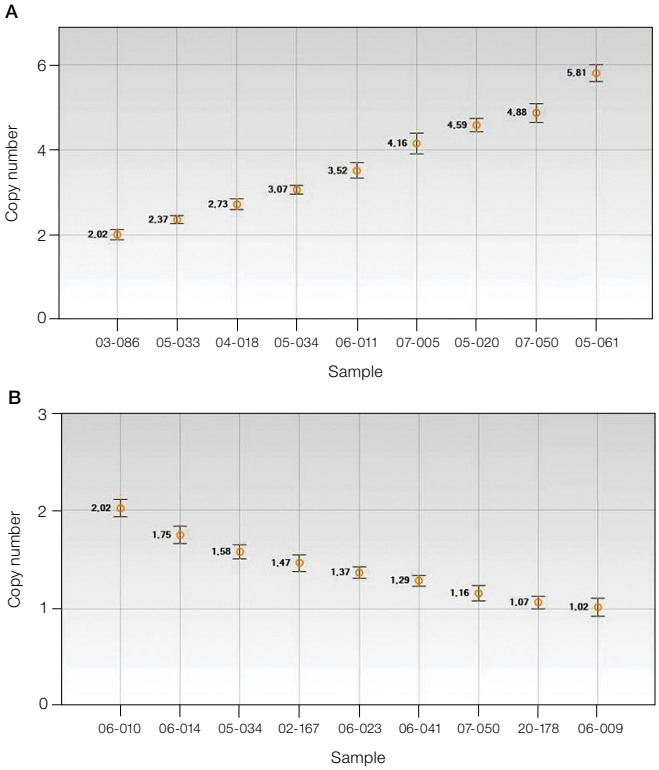
Uveal melanoma is a rare disease that results in metastatic spreading to the liver in up to half of the patients. Once liver metastasis is detected, life expectancy is limited to 6–12 months. Though little is known about the molecular pathogenesis of uveal melanoma, sensitive and specific molecular markers exist to predict disease outcome. Molecular aberrations, such as monosomy of chromosome 3 and gain of chromosome 8q, provide an accurate prognosis and are routinely analyzed with karyotyping and microarray approaches. However, these are time- and tissue-consuming methods. Alternatives for these methods are therefore evaluated.

Application

Using copy number variation (CNV) analysis, we have reanalyzed a series of 66 uveal melanomas that were previously analyzed with single nucleotide polymorphism (SNP) arrays. CNV assays were selected for four recurrent genomic aberrations in uveal melanoma. *PPARG* on chromosome 3p25, *NEDD9* on chromosome 6p24, *PTK2* on chromosome 8q24, and *NFAT5* on chromosome 16q22 were used in combination with *TERT* (5p15) as reference. Issues that can hamper tumor analysis are tumor heterogeneity due to disease progression and genetic mosaicism. With the application of Droplet Digital PCR (ddPCR™) we hoped to improve detection of malignant uveal melanoma cells.

ddPCR Results

Four recurrent genomic aberrations were studied in 66 uveal melanomas by CNV analysis using ddPCR. In two instances an accurate call could not be made because of abnormalities that included chromosome 5p15, in which the reference gene (*TERT*) in this test was located. For the remainder, a comparison with SNP array analysis revealed a good concordance for the DNA copy number. In one heterogeneous tumor sample, SNP analysis was indecisive for chromosome 3 status while CNV analysis indicated a loss of chromosome 3 in 25% of the cells. Fluorescence in situ hybridization (FISH) on isolated nuclei indicated a loss of chromosome 3 in 17% of the cells and thereby corroborated the copy number call made with ddPCR.



***PPARG* (A) and *PTK2* (B) are markers for loss of chromosome 3 and gain of chromosome 8q in uveal melanoma.**

BIO-RAD

Conclusions

By applying CNV markers at prognostic chromosomal locations we were able to test 66 uveal melanomas in a time- and tissue-efficient way. The quantitative nature of ddPCR allowed us to establish the degree of tumor heterogeneity. Moreover, heterogeneous tumors were more easily typed with ddPCR compared to SNP analysis, and this could be validated with FISH analysis.

By applying CNV assays using ddPCR we were able to achieve a very fast molecular prognosis for uveal melanoma. After inclusion of multiple stable references, application of this approach in the clinic would improve care for patients with uveal melanoma.

"After validation of genome analysis with ddPCR, digital PCR should be validated in a prospective study for use in clinical testing."

Publications

Maat W et al. (2008). Pyrophosphorolysis detects B-RAF mutations in primary uveal melanoma. Invest Ophthalmol Vis Sci 49, 23–27.

Maat W et al. (2007). The heterogeneous distribution of monosomy 3 in uveal melanomas: Implications for prognostication based on fine-needle aspiration biopsies. Arch Pathol Lab Med 131, 91–6.

Visit bio-rad.com/N/ddPCRSuccessVelden for more information.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 11 3065 7550* ***Canada*** *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 9532 220* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *026 674 55 05* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *1800 88 22 88* ***United Kingdom*** *020 8328 2000*

Bulletin N6556 Rev A US/EG *14-0574 0514 Sig 1213*