Correction

Correction: Minimal contribution of ERK1/2-MAPK signalling towards the maintenance of oncogenic GNAQ^{Q209P}-driven uveal melanomas in zebrafish

Mai Abdel Mouti[1], Christopher Dee[1], Sarah E. Coupland[2], Adam F.L. Hurlstone[1]

[1]Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom

[2]Department of Molecular and Clinical Cancer Medicine, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

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This article has been corrected: Due to errors in figure preparation, a transgenic line expressing an oncogenic allele of NRAS was mistakenly presented in Figure 1 as Q61L, whereas the proper allele is G12D. The corrected Figure 1 is shown below. The authors declare that these corrections do not change the results or conclusions of this paper.

Further, we observed hyperplasia of cutaneous melanocytes only and not uveal melanocytes expressing oncogenic NRAS^{G12D} or BRAF^{V600E} (Figure 2), in keeping with previous reports of cutaneous melanoma in zebrafish expressing oncogenic RAS or BRAF (Dovey et al 2009, Michailidou et al 2009, Patton et al 2005).

Generation of transgenic zebrafish expressing GNAQ^{Q209P} and NRAS^{G12D}

Recombinant plasmids encoding GNAQ^{Q209P} and NRAS^{G12D}

A NRAS^{G12D} ME vector was created by amplifying NRAS^{G12D} cDNA from a plasmid kindly supplied by Dr Piero Crespo (IBBTEC Inicio - Universidad de Cantabria) using primers with *att*B sites and then recombining with pDONR221 by performing a BP reaction according to the manufacturer's instructions.

In vitro three fragment recombination reaction between the entry clones (p5′E-mitfa promoter, pME-GNAQ^{Q209P} or pME NRAS^{G12D}, and p3′E-polyA) and Tol2-pDest-cryaa:venus destination vector was catalyzed by LR clonase II Plus enzyme mix (Life Technologies) according to the manufacturer's instructions to generate Tol2-pDest-*cryaa*:venus;*mitfa*:GNAQ^{Q209P}-polyA and Tol2-pDest-*cryaa*:venus; *mitfa*: NRAS^{G12D}-polyA recombinant expression plasmids.

We would like to thank Dr Helen Young in the Hurlstone laboratory for construction of Tol2-pDest-*cryaa*:venus; *mitfa*: NRAS^{G12D}-polyA.

Figure 2: Selective proliferative response of choroidal melanocytes to oncogenic GNAQ^{Q209P} expression and cutaneous melanocytes to oncogenic BRAF^{V600E} and NRAS^{G12D} expression. H&E staining of transverse sections through the eye of wild-type (A.I), Tg *mitfa*:BRAF^{V600E} (A.II), Tg *mitfa*:NRAS^{G12D} (A.III) and Tg *mitfa*:GNAQ^{Q209P} (A.IV) adult zebrafish demonstrate the specific association of choroidal thickening with oncogenic GNAQ^{Q209P} expression. Scale bar = 100mm. H&E staining of transverse sections through zebrafish torso show hyperproliferation of cutaneous melanocytes in transgenics expressing BRAF^{V600E} (B.II) and NRAS^{G12D} (B.III) as compared to wild-type (B.I) but not in GNAQ^{Q209P} (B.IV). Scale bar = 100mm.

Figure 3: Activation of ERK in cutaneous lesions driven by NRAS^{G12D} or BRAF^{V600E} but not uveal lesions driven by GNAQ^{Q209P}. IHC staining revealing pERK1/2 (readout of ERK activation) in representative hyperplastic lesions as indicated (**A**–**C**). Scale bar = 20 mm.

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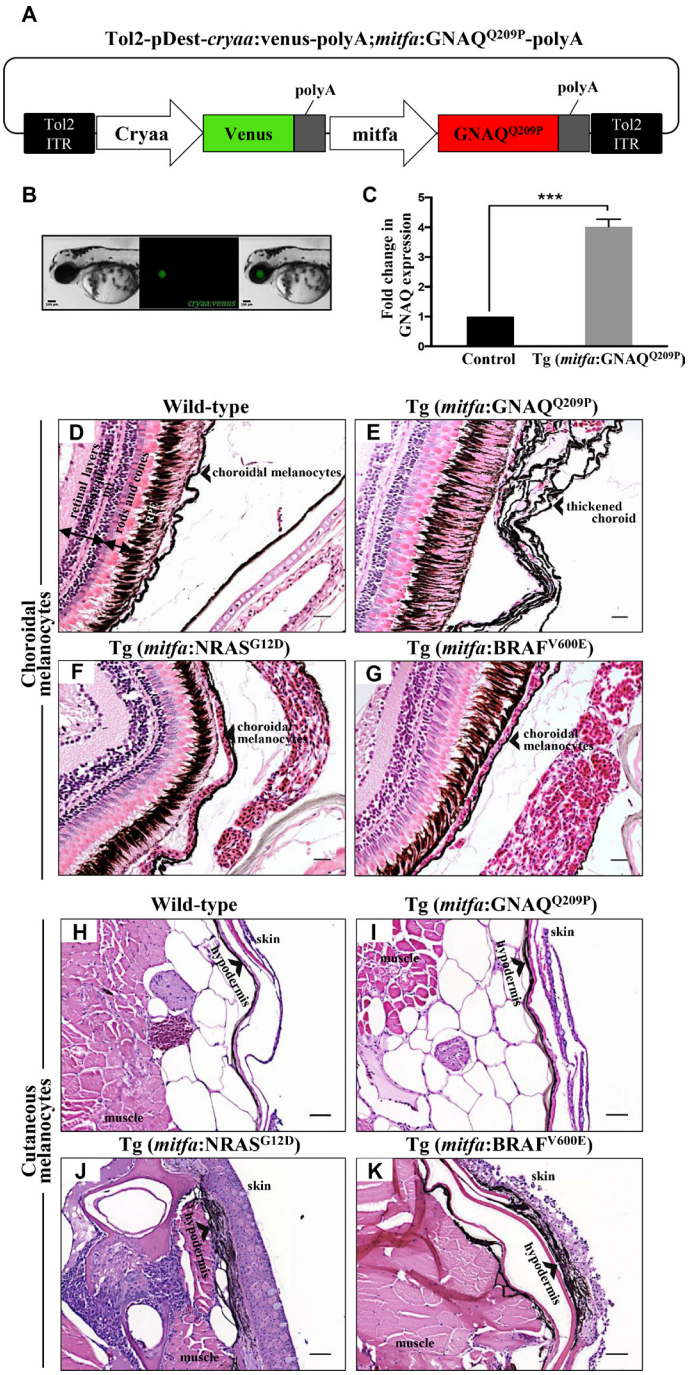

Figure 1: Only oncogenic GNAQ^{Q209P} is sufficient to induce choroidal melanocyte hyperplasia. (**A**) Schematic representation of elements in the Tol2-based transposon vector driving the expression of oncogenic GNAQQ^{209P} under the control of zebrafish *mitfa* promoter in the melanocyte lineage and Venus fluorescent reporter under the control of *cryaa* promoter in the eye lens. Abbreviations: ITR, inverted terminal repeat. (**B**) Example of a 5 dpf transgenic zebrafish embryo with a fluorescent eye lens. Scale bar, 100 µm. (**C**). RTqPCR data showing a 4.1 fold increase in GNAQ expression in the melanocytes of 2-month-old F_1 Tg (*mitfa*:GNAQQ^{209P}) zebrafish, as compared to non-injected controls. Data represents mean ± SEM of triplicates of three independent experiments. *** P <0.05 using twotailed, unpaired t test. (**D**, **E**, **F**, **G**) H&E staining of transverse sections of formalin-fixed and paraffin-embedded eye specimens of control wild-type, Tg (*mitfa*:GNAQ^{Q209P}), Tg (*mitfa*:NRAS^{Q61L}), and Tg (*mitfa*:BRAF^{V600E}) zebrafish, respectively. Choroidal hyperplasia observed in the thickened choroid (E; black arrowhead) was only observed in transgenic animals expressing oncogenic GNAQ^{Q209P}. In contrast, as compared to control wild-type (**H**) and Tg (mitfa:GNAQQ209P) (**I**) hyperplasia of cutaneous melanocytes (black arrowhead) was only detected in transverse sections of the torso region of Tg (*mitfa*:NRAS^{Q61L}) (**J**).and Tg (*mitfa*:BRAF^{V600E}) (**K**) zebrafish. Abbreviations: RPE, retinal pigmented epithelium. Scale bars, 20 µm.