

**Final Report**

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**Project title:** Validation of Molecular Targets Characterizing Invasive Retinoblastoma

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£39,756 (£13,252/year)

**Amount claimed to date:** £39,756

**Aims / Objectives of project:**

Although most retinoblastoma tumors are caused by mutations in the *RB1* gene resulting in lack of function of the RB1 protein, not all of the tumors behave the same way. Some tumors have the potential to invade the choroid and become metastatic whereas other tumors grow but remain entirely within the ocular globe. The aim of our proposal was to examine candidate genes identified using state-of-the-art technology to explore differences between clinically defined non-invasive tumors and invasive tumors with metastatic potential with the eventual goals of using these differences to increase the understanding of the process of malignant transformation and to define some of the differences between invasive and noninvasive retinoblastomas.

**Summary for CHECT scientific advisory board:**

Single-end RNA sequencing combined with a variety of sophisticated analytical tools was used to develop an accurate picture of the transcriptional activity of the cell. Comparison of profiles generated from invasive versus non-invasive tumors should allow a determination of candidate proteins and the pathways that contribute to the individual phenotypes of these tumors. We chose to investigate SKAP2, a gene that codes for a protein product that inhibits actin polymerization and thereby negatively regulates tumor invasiveness, and PDE1C, a member of the phosphodiesterase family that regulates cyclic nucleotide metabolism and thereby influences cell proliferation and differentiation.

1. A lentiviral vector that delivers a shRNA capable of reducing (knocking down) expression of the gene *SKAP2* was constructed. The endogenous expression of SKAP2 in the established retinoblastoma cell lines Y79, which forms aggressive invasive tumors in a murine xenograft model, and Weri Rb, which forms localized non-invasive tumors in a murine xenograft model was verified by western blot analysis before they were transduced with the constructs and used for *in vitro* assays. The knock-down was confirmed using quantitative PCR and the cells were compared using the *in vitro* cell adhesion and migration assays. Transduction of the Weri and Y79 retinoblastoma cells with the shSKAP2 construct significantly increased rates of proliferation relative to wild type (Rb+/+) controls or cells transduced with an empty lentiviral vector. The reduction of SKAP2 expression resulted in increased cell proliferation in either invasive or non-invasive retinoblastoma cells as well as in Rb positive control cells. Our future goals will include examining how SKAP2 knock down will affect cell migration *in vitro* or invasion and metastasis *in vivo* in the murine xenograft models of retinoblastoma.
2. One of the candidate genes that was overexpressed in invasive retinoblastoma cells including Y79 retinoblastoma cells coded for an enzyme involved in cyclic nucleotide metabolism. We therefore decided to investigate cyclic nucleotide phosphodiesterase (PDE) activities in Y79 cells in order to determine the role that these enzymes might play in the proliferation and invasiveness of retinoblastoma. We have presented evidence of the possible involvement of PDE1C, a calcium-calmodulin dependent cGMP phosphodiesterase, in the proliferation of Y79 retinoblastoma cells. Inhibition of PDE1C activity by pharmacologic small molecule antagonists resulted in the death of these cells. We have used 3 PDE1C-specific shRNAs as well as a non-silencing shRNA and inserted them into pGIPZ vectors to show that the specific knockdown of PDE1C gene expression with shRNA also prevents the growth of these cells. The 4 different lentiviral vectors (1 control and 3 PDE1Cs) have been prepared and are being used to verify reduction of PDE1C expression. We will then separately transduce the viral particles into the retinoblastoma cells to determine growth characteristics and phenotypic properties *in vitro* and *in vivo*.

With the support of CHECT, we have identified two gene products that are differentially expressed in invasive and non-invasive retinoblastoma tumors. These gene products have opposite effects on proliferation of retinoblastoma cell lines *in vitro* and may have the potential to influence the phenotypic behavior of retinoblastoma tumors. Further studies of SKAP2 and PDE1C protein levels in retinoblastomas may reveal new ways to predict the metastatic potential of the tumor and influence counseling and treatment options.

**Lay summary for CHECT general committee:**

The RB1 protein is a master regulator of cell growth. Changes (mutations) in the *RB1* gene that codes for this protein are known to be the primary cause of most retinoblastomas (ocular tumors) detected in children and have also been found in common adult cancers such as lung, breast, prostate and ovarian tumors that have metastasized. Although almost all children with retinoblastoma have mutations in the *RB1* gene, not all children have invasive disease, a precursor to the development of metastatic disease. We hypothesized that by comparing gene expression in tumors derived from children with invasive retinoblastoma to tumors derived from children with non-invasive retinoblastoma, we could gain an understanding of the metabolic steps after the mutation of *RB1* that lead to tumor invasion and subsequent metastatic disease. We have studied gene expression in both invasive and non-invasive retinoblastoma tumors using single end RNA-Seq technology and have identified candidate genes that are more highly expressed in invasive retinoblastoma tumors when compared to non-invasive retinoblastoma tumors. We chose to study two of these candidate genes by examining differences between invasive and non-invasive retinoblastoma tumors using retinoblastoma cell lines that retain their original phenotype (invasive or non-invasive behaviors) when used to induce tumors *in vivo*. The results obtained have identified potential therapeutic targets to treat retinoblastoma.