Introduction
Hedgehog (HH) is an evolutionarily conserved pathway in vertebrates that is vital in organ patterning and the maintenance of cellular polarity during embryogenesis. Increasingly, aberrant HH signalling has been reported in a number of malignancies. Most notably, basal cell carcinoma (BCC) of the skin, in which mutations of the HH receptors (Smoothened or Patched) result in a constitutively activated HH pathway, characterised by the nuclear translocation of the Gli transcription factors. We have previously shown that the epithelial-specific integrin \( \alpha v \beta 6 \) is up-regulated in a variety of solid tumours, including morphoeic (infiltrative) BCC. The integrin is expressed during issue remodeling, fibrosis and cancer but is not present in normal adult epithelia. However, the mechanisms behind the regulation of \( \alpha v \beta 6 \) expression remain unclear.

Aim
The aim of this project is to investigate the regulatory mechanisms behind \( \alpha v \beta 6 \) integrin and its potential interaction with the Hedgehog signalling pathway.

Summary of Research Milestones
We have made significant progress in this research project, particularly the laboratory-based experimental side (which the Small Grant has been instrumental in). Below is a brief summary of some of our results:

1. We have carried out both immunohistochemical analysis on tissue microarrays from basal cell carcinoma (BCC) and pancreatic carcinoma (PC) cases, showing an inverse correlation between \( \alpha v \beta 6 \) expression and Gli1 expression. (60 BCC cases and 62 PC cases; Figure 1).

![Figure 1. IHC panel showing inverse pattern of expression of Gli1 and \( \alpha v \beta 6 \) expression.](image)
2. We have carried out functional studies (three-dimensional organotypic cultures), where we have observed that Gli1 significantly down-regulates $\alpha v \beta 6$-dependent functions.

![Figure 2](image)

Figure 2. Three-dimensional organotypic cultures showing that Gli1 significantly downregulates invasion (i), Western blotting confirming Gli1 silencing, which restored invasion. Invasion was quantified using ImageJ software (iii).

3. We have observed that Gli1 significantly down-regulates $\beta 6$ gene expression using quantitative real-time PCR techniques. We introduced Gli1 into a second skin cell line (HaCaT), to confirm our findings in the NTert skin cell line (included in preliminary data). These results confirmed that Gli1 had an inhibitory effect on $\beta 6$ transcript levels.

![Figure 3](image)

Figure 3. qRT-PCR showed that Gli1 significantly downregulated $\beta 6$ mRNA levels in both the NTGli1 (i) and HaCaT Gli1 (ii) cell lines. Expression was restored upon Gli1 silencing by siRNA.

**Future Work**

1. To dissect the mechanism of Gli1/$\alpha v \beta 6$ expression, we have preliminary results showing that the master regulatory mechanism behind $\alpha v \beta 6$ transcriptional regulation is via TGF-$\beta$ signalling. We are currently investigating the convergence of that pathway with Gli1 signalling.

2. To consolidate the preliminary Luciferase assay results using our promoter constructs for ITGB6 gene.

**Biological and Clinical Significance**

Hedgehog inhibitors are currently in clinical trials, but have shown equivocal results. Our findings may provide a biological explanation for the failure of some Hedgehog inhibitors, and re-emphasise the need for stringent patient stratification in clinical trials. These findings are also of biological significance, as we describe novel cross-talk between Hedgehog and integrin signaling in cancer.

**Dissemination and Presentations**

Data from this project as funded by this scheme has been presented nationally and internationally. Most notably, I presented this work at the
Japanese Society for Pathology annual meeting in June 2013. I was selected by the Trainee Subcommittee to present in the meeting and The Pathological Society sponsored me for the meeting. This was as a result of my winning the Plenary Session prize when I presented the preliminary results from this project in Sheffield at the PathSoc summer meeting in 2012.

**Publication plans**
This work is now in the process of being finalised for submission for publication; and the Pathological Society will of course be acknowledged.

**Further funding**
I intend on applying for further funding to investigate other arms of this project (particularly the pancreatic cancer findings, results of which I could not include here due to lack of space.)