RCPA Foundation Final Report (Dr Andrew Colebatch)

Project title:

The distribution and evolution of telomerase reverse transcriptase (*TERT*) promoter mutations in primary cutaneous melanoma

Background:

Mutations in the promoter region of the *TERT* gene are the commonest somatic mutation present in cutaneous (non-acral) melanoma, present in 50-80% of cases (Huang et al. Science 2013, Heidenreich et al. Nature Communications 2014, Griewank et al. JCNI 2014). In 80% of cases, this mutation occurs at either the -124 position (basepairs upstream of the transcription start site) or the -146 position. Given the role of TERT in maintaining telomere length, this mutation is likely to be a key step in melanoma tumourigenesis, at least partially conferring replicative immortality to presumptive melanoma cells. However the study of early lesions and *TERT* mutational heterogeneity in melanoma is very limited. To date only a single study has evaluated *TERT* status in melanocytic nevi, finding that all 25 were negative for the typical mutations (Horn et al. Science 2013). This is potentially a key difference with *BRAF* mutations, another critical melanoma mutation present in approximately 50% of cutaneous melanomas. *BRAF* mutations are present in around 80% of melanocytic nevi, and therefore cannot be used to ascertain malignant potential, e.g. melanocytic nevus versus nevoid melanoma. This difference suggests that *TERT* promoter mutations are acquired later in the step-wise model of melanomagenesis from nevus to fully fledged malignancy, and begs the question as to when during development this mutation occurs. The study of melanoma

may shed light on this, in that a proportion of cases demonstrate histologically distinct compartments which are thought to correspond to temporally distinct phases of growth.

This project has two goals. The first is clinical diagnostics. If *TERT* mutations are indeed confined to certain compartments of clearly invasive melanoma, suggesting that *TERT* promoter mutation is a critical component of the invasive phenotype, then mutational testing of either borderline melanocytic lesions or lesions with questionable invasion becomes diagnostically informative. The sensitivity of the ddPCR assay should allow the detection of very small populations of fully invasion-competent melanoma cells, and may permit stratification of lesions into risk categories with appropriate clinical monitoring. Together with future prospective datasets looking at the natural history of *TERT* positive melanocytic lesions of uncertain malignant potential, this study will contribute to the development of *TERT* promoter mutation screening as a clinical biomarker for melanoma. Moreover, this ddPCR methodology will be of use in other scenarios in which DNA is limited, such as circulating tumour DNA. In this case, it will be especially attractive to follow patients which have melanomas that lack classic oncogenic mutations in *BRAF* and *NRAS* (accounting for approximately 50% of Australian melanoma patients).

The second goal concerns fundamental melanoma tumour biology. Determining the distribution of *TERT* promoter mutation in different architectural regions of a melanoma may reinforce the stepwise model of melanoma tumourigenesis, and suggest a critical target for future therapeutic development. If *TERT* promoter mutations are requisite for the full malignant potential of melanoma, then its use as a diagnostic marker becomes viable and the need for therapeutic approaches to inhibit *TERT* become pressing.

Objectives and outcomes:

1. Design of a highly sensitive *TERT* promoter mutation ddPCR assay

   Separate ddPCR assays were designed for each of the two most common *TERT* promoter mutations (-124 and -146). Limit of detection testing demonstrated accurate determination of mutant allele fraction down to 0.04% for both assays. Moreover by analysing cell lines we demonstrated that ddPCR permits a more accurate assessment of *TERT* mutant allele fraction. This was achieved by using novel PCR additives which will be of potential use in other GC-rich regions. As such, this project has resulted in the development of a novel *TERT* promoter mutation detection assay which will have potential clinical utility.

2. Determination of the timing of *TERT* promoter mutations during melanomagenesis

   Microdissection of nineteen cutaneous melanoma samples was performed, yielding matching in situ and invasive melanoma regions for each sample. DNA was extracted from each region and ddPCR performed. Fourteen samples had *TERT* promoter mutations, of which twelve had matching mutations between in situ and invasive regions, suggesting that *TERT* promoter mutations are an early event in melanoma development. Interestingly, two samples had additional *TERT* promoter mutations in single regions, suggesting the presence of distinct subclones in some melanomas. This in turn suggests that a linear progression from in situ to invasive melanoma may be an overly simplistic model of melanoma development.

Conclusion and future directions:

The successful design of a *TERT* promoter mutation ddPCR assay has several ramifications. The ddPCR test is far more sensitive and permits more accurate estimation of mutant allele fraction than the current gold standard of Sanger sequencing. Moreover it is relatively inexpensive and has a short turnaround time compared to next generation sequencing approaches. As such, it should be considered as the preferred method of analysing *TERT* promoter in clinical material. Analysing the *TERT* promoter for mutations may be of use in characterising melanocytic lesions with borderline malignant features, and studies evaluating such lesions with long term follow up will assist in its development as a prognostic biomarker.

Moreover, the ddPCR technique is not limited to any one particular sample type or even malignancy. *TERT* ddPCR would be potentially of use in the setting of evaluating circulating tumour DNA in *BRAF/NRAS* wildtype melanoma patients. Moreover given the frequency of *TERT* promoter mutations in urothelial carcinoma, ddPCR of urine is another possible use of this assay.

Finally, this project demonstrated that *TERT* promoter mutations are acquired early in the development of melanocytic lesions, consistent with other published work (Shain et al. NEJM 2015). We have extended this finding by demonstrating the presence of occasional subclones defined by alternate *TERT* promoter mutations in melanocytic neoplasms, which in turn suggests that a sequential linear progression from nevus to invasive melanoma is overly simplistic.

Non-technical summary:

Melanoma is the most fatal skin cancer, and understanding its evolution is important for accurate diagnosis and treatment. Melanoma, like other cancers, acquires mutations as it develops. In some melanomas, pathologists can observe under the microscope different areas which represent less aggressive and presumably earlier in development as well as more aggressive and more malignant biology and behaviour. In this project, we looked for mutations in the control regions for a gene called *TERT*, which is involved in immortalising melanoma cells, a critical step in the development of cancer. These mutations have recently been shown to be present in 50-80% of melanomas, making this the commonest mutation in melanoma.

First we designed a very sensitive test to detect these *TERT* mutations, after which we used this test to look at the presence of mutations in different areas of melanoma. We showed that *TERT* mutations occur early in the development of melanoma, and could therefore possibly be used as a test to detect early cancer. This may assist pathologists to better diagnose difficult melanomas.

Publications from this project:

Colebatch AJ, Witkowski T, Waring PM, et al. ‘Accurate detection of TERT promoter mutations using droplet digital PCR’ [In preparation]