Development of Merkel Polyoma Virus detection assays for accurate diagnosis of Merkel Cell Carcinoma

NETwork! New Zealand Society for Oncology

Braden Woodhouse
b.woodhouse@auckland.ac.nz

Braden Woodhouse[1,2], Tamsin Robb[2], Kate Parker[1], Rose Miller[3], Greg Hayward[4], Paul Restall[5], Benjamin Lawrence[1], Michael Findlay[1], Cristin Print[2], Cherie Blenkiron[2,6,7]

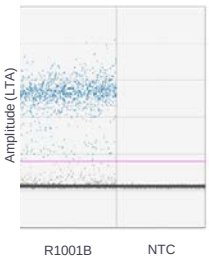
[1]Discipline of Oncology, FMHS, University of Auckland, New Zealand, [2]Department of Molecular Medicine and Pathology, FMHS, University of Auckland, New Zealand, [3]Anatomic Pathology Service, Auckland District Health Board, New Zealand, [4]Waitemata District Health Board, [5]LabPLUS, Auckland District Health Board, [6]School of Biological Sciences, FoS, University of Auckland, New Zealand, [7]Department of Obstetrics and Gynaecology, FMHS, University of Auckland, New Zealand

Droplet Digital PCR (ddPCR)

ddPCR generates many reactions (<20,000) within a single 20µL well, partitioning droplets using water oil emulsion. It then measures the absolute number of (nanoliter-sized) droplets containing the target molecule giving a discrete measurement of positivity. After setting the threshold based on the no template control (NTC), the data can then be analysed based on the number of positive droplets, to determine the original target concentration.

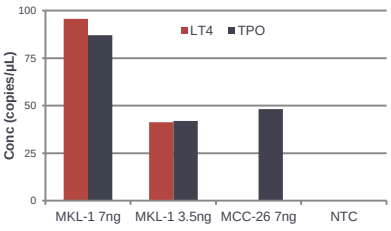
ddPCR has many advantages over other forms of PCR:

- Provides absolute count of target copies per input sample – no need for standard curves
- High precision – small fold copy number differences reliably detected due to massive sample partitioning
- Less reagent required – lower experimental costs
- Higher signal-to-noise ratio
- Smaller sample requirement preserving precious samples

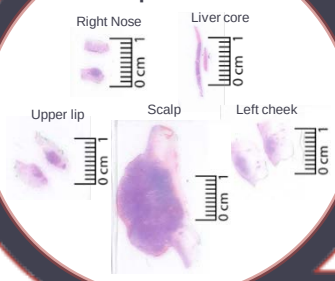


Cell Lines

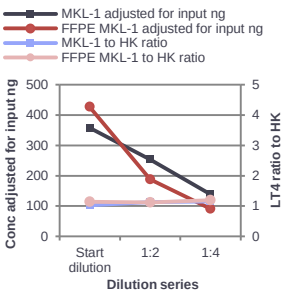
- MKL-1 – MCPyV+ positive, confirming reports of ~2 copies/cell (normalised to TPO)
- MCC-26 – MCPyV- negative, LoH suggested in genomic location for TPO, based on comparative genomic hybridisation (CGH) data analysis (1)



Small specimen size



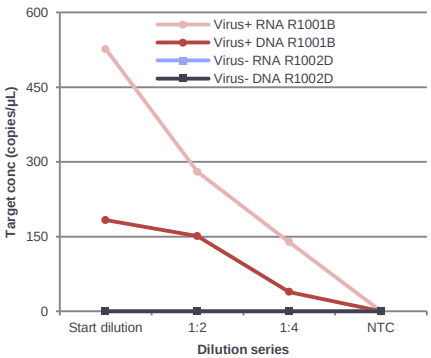
Linearity in cell lines



Multi-method detection

When using the Merkel Virus (MCPyV) primer, clear correlation between the DNA and RNA is seen for virus detection. A virus positive and virus negative example is shown, with both DNA and RNA demonstrating evident viral positivity in sample R1001B and no virus detected in sample R1002D using both DNA and RNA.

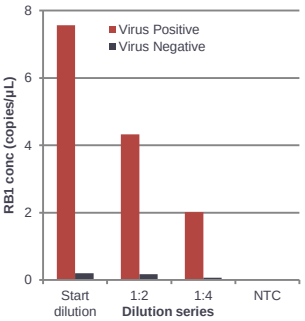
In progress currently, is the work-up of methods for immunohistochemistry (IHC), where the aim is for each sample to be tested for viral status and validated by the three methods of detection: DNA, RNA and protein. Protein detection by IHC is particularly valuable for tiny specimens such as punch biopsies.



RB1 increased in virus positive

Whilst the exact mechanism of MCPyV's involvement in MCC tumorigenesis is still being discovered, it is thought to involve the retinoblastoma protein (Rb). Studies have proposed that an LTAg mutation results in formation of a protein that induces a conformational change in Rb, preventing it from carrying out it's usual tumour suppressor function, contributing to cancerous cell transformation.

There are a few studies that suggest higher *RB1* expression in MCPyV positive tumours, and lower *RB1* expression in virus negative tumours (2, 3, 4). Our results thusfar appear to correlate well with these previous publications, with an apparent correlation between viral status and *RB1* expression, suggesting that *RB1* expression could be used as a surrogate marker for viral status.



Summary and challenges

- Comparison between cell line and FFPE samples showed very similar overall counts per input amount, demonstrating that despite the damaging fixation process, analysis from FFPE tissue specimens is still highly representative of Merkel tumours.
- As this is a cancer predominantly diagnosed in elderly (5), it is not uncommon for small tumours to remain *in situ* unresected after an initial biopsy has been taken. Additionally, many patients present with very small skin tumours in comparison to other cancers, and as such most Merkel cell tumours have very little sample tissue. With a combination of ddPCR (very sensitive to low DNA input - we have detected virus presence as low as 0.2ng) and IHC detecting the protein, this will enable accurate detection of virus in the smallest of tumours.
- Neuroendocrine tumours are known to be highly aneuploid, as found in the MCC-26 cell line where one copy of TPO has been lost. This presents an interesting challenge in selecting suitable stable housekeeper genes in these tumours.
- It is proposed that viral-mediated tumorigenesis directly involves *RB1*, whereas virus-negative tumours are thought to involve *TP53* (6), likely by a UV-mediated mechanism. This is particularly of interest to our NZ cohort, where we have seen a much lower incidence of viral-positive tumours, influencing diagnosis and potentially treatment options.

References

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2. *Bhatia et al* 2010 PMID: 19551862
3. *Sihto et al* 2011 PMID: 21642382
4. *Harms et al* 2013 PMID: 23223137
5. *Robertson et al* 2015 PMID: 25801524
6. *Waltari et al* 2011 PMID: 20949558

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