

Development of a cheap and simple immuno fluorescent staining protocol for Mismatch repair proteins in possible HNPCC related tumours.

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disorder. Patients suffering from this disorder are at high risk for developing colon cancer and tumours in the endometrium, small bowel, pancreas, biliary tract, stomach, ovary, and urinary tract. There is no molecular or diagnostic protocol available for HNPCC but patients are usually diagnosed by the so-called Amsterdam criteria. Almost all tumours of patients with HNPCC demonstrate mild or high microsatellite instability (MSI). MSI is the result of small deletions or insertions at mono- or dinucleotide genomic sequences. These mutations are caused by a defective DNA Mismatch Repair (MMR) system which will normally avoid 'strand slippage' during replication. Germline mutations in five genes (*MSH2*, *MLH1*, *PMS1*, *PMS2*, and *MSH6* [also designated "*GTBP*"]), all coding for MMR proteins, have been found.

- Archived tumour samples are being tested for the presence or absence of expression of three MMR proteins (*MLH1*, *MSH2* and *MSH6*) by immuno fluorescence. It has been shown previously that the absence of one of these three proteins is 90% concordant with the presence of a high MSI phenotype. In doing this we can test whether there is a higher proportion of HNPCC related tumours in the Riverina compared with other areas of NSW. Previous work has shown that the proportion of MSI colorectal tumours in the Riverina is as high as 35% of all colorectal tumours (Weidenhofer & Kalle, unpublished results). By performing these tests on a larger and more diverse group of tumour samples we can set up the immune fluorescence labelling design and at the same time substantiate these findings.
- By enhancing the immunofluorescence protocols for the detection of the three previously mentioned MMR proteins we can develop a triple staining to detect protein expression in tumour and normal samples. This would enable us to determine protein expression by fluorescent ratio measurements and determine the possible MSI status of the sample in a relative easy and cost effective way.
- Using the ratio measurements would enable us to measure protein expression by the aid of immunofluorescence in a large sample of normal and tumour tissues. If we can detect decreases in expression of one of the three MMR proteins (for example due to haplo-insufficiency) before tumours have been diagnosed we would have developed a test with immediate clinical and diagnostic implications

Progress up to date has been in the immunostaining area. Mono and double staining protocols have been developed. Current efforts are on collecting samples and developing the triple staining model. Work will be finished by June 2007

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