Assessment of a putative oncogene ZNF217 within amplicon 20q by multiplex quantitative real-time PCR.

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Taqman PCR using the ABI7700 (PE Applied Biosystems). Multiplex quantitative real-time PCR was used to amplify internal control gene, *Beta globin* and the candidate gene, *ZNF217* simultaneously in the same well. For each case, *ZNF217* gene copy number was then calculated using the values of the mean C_t of *ZNF217* (each case was assayed in triplicate) minus the C_t of *Beta globin*. The tumour values of C_t *ZNF217* – C_t *Beta globin* were compared to those of normal diploid colon mucosa (n=10) and colorectal cell lines (n=6) for which *ZNF217* had been independently established using *FISH*, and assigned a specific copy number. Of the 81 tumours (42 Dukes' B and 39 Dukes' C) assessed, 18 were diploid, 15 had loss of gene copy and 48 contained some level of amplification at the *ZNF217* locus. Chi-square analysis found no significance in the distribution of *ZNF217* gene copy number between Dukes' B and Dukes' C tumours. In this study we found that ZNF217 amplification is a frequent event in colon cancer (48/81 tumours = 59.3%) and that the extent of its amplification varies markedly between tumours (range 3 - 13 copies).

1. Rooney PH, Murray GI, Stevenson DAJ, Haites NE, Cassidy J, McLeod HL. 1999 *Br J Cancer* **80**:862-873.

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ASSESSMENT OF A PUTATIVE ONCOGENE *ZNF217* WITHIN AMPLICON 20q BY MULTIPLEX QUANTITATIVE REAL-TIME PCR.

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Detection of gene amplification is a recognised process through which oncogenes can be identified. In this study we have coupled two powerful techniques, laser capture-microdissection and multiplex quantitative real-time PCR to quickly and accurately assess the gene copy number of candidate oncogene *ZNF217* in colon cancer. Several studies of colorectal cancer using comparative genomic hybridization (CGH) have identified chromosomal arm 20q, as the region most commonly gained in this tumour type[1]. In addition high density *FISH* mapping analysis of the 20q amplicon in colorectal cell lines within our laboratory has identified *ZNF217* to be contained within a region, 20q13.2 of high-level amplification. In this study the frequency and level of *ZNF217* amplification in 81 colon tumours was assessed by multiplex quantitative real-time PCR. Tumour cells were isolated from unfixed 10µm sections of original snap frozen tumour, using a PixCell II laser capture microdissection system (Arcturus Engineering). Tumor tissue was identified and removed by the laser to a microdissection cap. Approximately 500 laser pulses were taken per tumor (laser settings spot diameter set at 15µm, pulse duration 5 milliseconds and power 100mW). Following protein lysis with 20mg/ml proteinase K, DNA was assessed by