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OC-103

ALTERED STEM CELL DYNAMICS IN HUMAN COLON ADENOMA CRYPTS ALLOW RAPID EXPANSION AND FIXATION OF MUTATIONS DURING CLONAL EXPANSION

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Introduction Methylation patterns at CpG islands within non-expressed genes are surrogate markers of cell ancestry and dynamics in normal human colon crypts, and have been used to provide evidence that crypts contain multiple stem cells within a niche.[1] Over time, a stem cell and its progeny will come to occupy the niche- *niche succession*, and subsequently the crypt- *monoclonal conversion*. Crypts divide by *crypt fission*, whereby a parent crypt produces two clonally related daughter crypts; this is the predominant method by which crypts expand in early tumorigenesis.[2] By analysing methylation patterns of normal human colon crypts, known to be related by their sharing clonal point mutations in mitochondrial DNA (mtDNA), we found that the rate of niche succession is slow (years) and crypt fission is rare.[3] There is a need to evaluate these dynamics in adenomas to understand how quickly they grow; we hypothesise that both stem cell niche succession and crypt fission occur at a faster rate in adenoma tissue enabling accelerated clonal expansion.

Methods Fresh frozen human adenomas were obtained and clonal patches of adenomatous crypts identified by combining enzyme histochemistry and laser capture microdissection with the use of PCR sequencing to demonstrate shared clonal point mutations in mtDNA. Using DNA from the same crypt, methylation patterns at CpG islands of three non-expressed genes were determined using clonal bisulphite sequencing.

Results In contrast to normal human colon, multiple, large clonal patches of crypts that shared common point mutations in their mtDNA were identified within human adenomas; these recently related adenomatous crypts had very similar, conserved methylation patterns compared to unrelated crypts ($p=8.08\times10^{-12}$).

Conclusion This study demonstrates that increased rates of stem cell niche succession, monoclonal conversion and crypt fission enable accelerated clonal expansion of mutated crypts

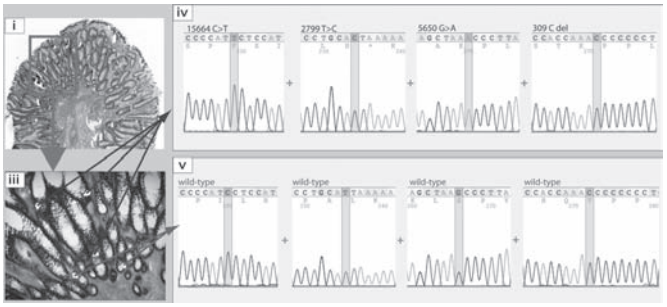

Figure 1 OC-103

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within human adenomas, suggesting that these up-regulated processes allow rapid fixation and spread of successive oncogenic mutations during early tumorigenesis; further data collection will allow the time course of these events to be estimated. Combining the techniques used here with clonal oncogenic changes may reveal the selective growth advantage of specific mutations within adenomas.

Competing interests None.
Keywords adenoma, dynamics, stem cells, tumorigenesis.

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