Title: *RNF43 G659 frameshift variants differentiate primary and metachronous Lynch syndrome associated colorectal cancers*

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Abstract

Metachronous colorectal cancers (CRC) arise in up to 50% of Lynch syndrome (LS) patients depending on the extent of the primary colectomy. We examined the genomic differences between 38 primary and 20 metachronous whole exome sequenced LS-associated colorectal tumours. Comparative analysis revealed the known tumour suppressor *RNF43* as a key enriched gene in the metachronous tumours (75% vs 34%, OR 5.6, 95% CI [1.5, 24.3]). Similar, but non-significant, trend was observed in a small tumour subset of 10 primary-metachronous pairs (70% vs 40%, OR 3.3 [0.4, 33.3].

Functionally, *RNF43* codes for a ubiquitin ligase acting as a tumour suppressor of Wnt pathway with a dual role as a negative regulator of PI3 kinase. *RNF43* contains a 7-nucleotide microsatellite prone to a frameshift (G659fs) that, unlike N terminus loss-of-function mutations, activates PI3K signalling but does not impair the Wnt tumour suppressor function [2][3].

Subgroup analysis confirmed non-G659fs (17/44), but not G659fs (27/44), mutations as a driver of Wnt pathway. Non-G659fs variants were mutually exclusive with key Wnt pathway genes *APC* ($p<0.0001$) and *CTNNB1* ($p<0.05$) across the cohort. G659fs variants did not significantly exclude *PTEN* or *PIK3CA* mutations. Moreover, it was revealed that G659fs is the sole subtype responsible for *RNF43* enrichment in metachronous tumours. This discrepancy may have its roots in the differing immune selection landscape of metachronous tumours and reflect a different sequence of accumulation of carcinogenic mutations at the early stages of carcinogenesis as opposed to the primary tumours.

Word count (excl. references): 240

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