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Low frequency of *MLL3* mutations in colorectal carcinoma

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Large-scale sequencing of cancer genomes holds great promise in identifying gene mutations that drive human cancer progression. A study was reported recently in which the coding region of 13,023 genes was systematically sequenced within a panel colorectal tumors [1]. Six somatic heterozygous mutations of the *MLL3* (Mixed-Lineage Leukemia 3) gene were identified in 35 colorectal tumors, making it the 4th highest mutation frequency in the disease [1]. *MLL3* had not been implicated previously in human cancer, however, the highly-related *MLL1* is a known proto-oncogene in myeloid and lymphoid leukemia [2]. MLL proteins are transcription factors that promote gene activation through histone methyltransferase activity performed by their conserved SET domain at the C-terminus [2]. Importantly, two of the identified mutations from the prior study (R1906stop and 1290del40nt) resulted in truncation of the MLL3 protein eliminating its catalytic SET domain [1]. Three mutations resided near the N-terminus within a stretch of PHD domains (C347G, D400N, L478W), which are known chromatin-binding modules [3]. Since most of these mutations would be predicted to impair MLL3 function, these findings raised the tantalizing possibility that *MLL3* mutations might play a role in the initiation and/or progression of the disease. It should be noted that considerable debate exists over the proper statistical methods used to distinguish passenger from driver status of mutations identified in such large-scale studies (see commentaries on [1]). This issue is particularly relevant to large genes (such as *MLL3*), which would be expected to contain a higher frequency of passenger mutations occurring at random in a given tumor. Hence, a reevaluation of the original mutation data using a more stringent filter for passenger mutation frequency deemed the identified *MLL3* mutations as not achieving statistical significance [4]. Based on these uncertainties regarding the true significance of *MLL3* mutations in colorectal carcinoma, it is imperative that the frequency of mutations be evaluated in an independent cohort of disease.

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In order to validate the mutation frequency of *MLL3* in colon cancer, we sequenced all 59 exons of *MLL3* (14.7 kb coding sequence) in an independent group of 57 colon tumor DNA samples (all processed from primary tumor specimens). Eighty-two PCR products spanning 867 kb of total tumor DNA were generated and subjected to direct sequencing in both orientations using Applied Biosystems 3730xl DNA Analyzer. Chromatogram analysis was performed using Consed software, which was verified by visual inspection. Nucleotide changes matching known SNPs were removed. All remaining nucleotide changes were then examined in matched normal control tissue DNA to examine somatic or germline status. All identified changes were verified by re-amplifying and sequencing the relevant PCR product.

Our analysis identified one somatic mutation of *MLL3* among 57 colon tumors (1.8%), encoding a heterozygous missense mutation, S3449F. Serine 3449 lies outside of all conserved domains within the *MLL3* protein, and the mutation is therefore of uncertain functional significance. Our study identified also several novel non-synonymous germline variants of *MLL3*, likely representing previously unidentified SNPs. The passenger mutation frequency in colon cancer has been estimated to be approximately 0.55 per Mb, although this number is likely to vary among distinct genomic regions and depend upon type and stage of the cancer [4]. Therefore, our single identified mutation within 867 kb of sequenced tumor DNA approximately matches the number of passenger mutations to be expected by chance.

While our findings do not support a high frequency of *MLL3* mutations in colon cancer, as was initially reported [1], our results are consistent with the subsequent reanalysis of the data using more stringent statistical evaluation of passenger mutation frequency [4]. It should also be noted that the original screen was performed largely in cell lines and xenografts derived from metastases, whereas our analysis was performed exclusively in primary tumors. It is possible that passenger *MLL3* mutations were more prevalent in the prior study due to prolonged cell incubations, whether in culture or following transplant into mice. Our findings are in concert with a prior sequencing study of 104 cases of colorectal carcinoma in which no mutations were identified in *MLL3* [5]. However, this study only examined 5 of the 59 exons of the *MLL3* gene. *MLL3* mutations might occur at higher rates in other human cancers, such as glioblastoma multiforme and pancreatic ductal adenocarcinoma [6]. Further insights into a possible role of *MLL3* in carcinogenesis would likely be gained from more expansive sequencing studies as well as through functional approaches.

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