A STUDY OF NEWER PROGNOSTIC EPIGENETICS MARKERS SFRP1 & IGFBP3 IN COLORECTAL CARCINOMA

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Summary

SUMMARY

Colorectal carcinoma (CRC) is one of the leading causes of mortality worldwide. It remains a worrying human health issue, which contributes about 8.2% cancer associated deaths globally. It is the third most commonly diagnosed cancer around the globe, and in India it was recorded as fourth and fifth most common cancer in males and females with incidence rates of 6.4%, 3.4% respectively. The development of metastasis in distant organs, such as liver, lungs etc is the main cause of death, thus ~40-50% of patients relapse and die of metastatic disease even after curative surgery. However, CRC can be cured 9/10 times if we detect this in time. Many biomarkers as developed to detect the CRC, carcinoembryonic antigen (CEA) is the 1st one and widespread used. Epigenetic alterations such as DNA hypermethylation, can be used for the early detection of pre-malignant lesions, including adenomatous polyps in the colon. Promoter hypermethylation of tumor suppressor genes thereby transcriptional silencing gained significance as prognostic markers in the CRC diagnosis, prognosis and therapy. Generally, more than half of CRC cases were diagnosed at stage II and III, and the mainstay of treatment option is only surgery. After surgery, patients survival depends on disease relapse because in most of the cases recurrence will happen. So, it becomes important to identify the high-risk patients who need additional adjuvant therapies besides the regular chemotherapy. In this study, our aim is to analyse CIMP status of colorectal cancer cases using and new panel of CIMP marker gene described by Lee, et al 2008. To determine CIMP status of CRC cases we analysed methylation status of five marker genes panel i.e. CACNA1G, IGF2, NUROG1, SOCS1, RUNX3 using methylation specific PCR and phenotype of these cases verified by histopathological analysis. If three or more than three genes found methylated case considered as CIMP high if less than three gene found methylated case considered as CIMP LOW. We analysed all five marker genes in all 56 cases and individual findings of all gene described below. In this study we analyzed the CIMP status in the stage II and stage III CRC cases and found that CIMP high status is significantly associated with poor survival. We found 44.6% CRC cases had CIMP-high where as 55.4% had CIMP low. In CIMP marker genes analysis we found CACNA1 gene was methylated in 37.5% cases, IGF2 gene was found methylated in 58% cases, NUEROG1 was methylated in 57% cases RUNX3 was found methylated 33.9% cases and SOCS1 gene methylation was found in 42.9%

cases. CACNA1G, SOCS1 are associated with poor survival but difference in survival was not significant. Methylation of a IGF2 gene doesn't show significant association with survival period however methylated NEUROG1 shows better survival. Though the survival period depends on many factors, a possible correlation was drawn by this study that CIMP high status means less survival in stage II CRC cases. There was no association observed in other clinicopathological factors such as age, gender, tumour stage, grading, histological type, dietary habits and alcohol intake.

Association of CIMP status with clinicopathological characteristics- To find the relationship between patients clinicopathological characters and CIMP status we perform chi square test. The results of chi square test shows the CIMP status was not associated with patients age, gender, **The** association of CpG island methylator phenotype was found with tumor site. We 70% cases of poorly differentiated tumor had CIMP high.

SFRP1 gene promoter methylation in tumor tissue- We analyzed SFRP1 gene promoter methylation status in tumor tissue and adjacent normal tissue. In 42 out of 56 (75%) CRC cases SFRP1 gene was methylated while in 14/56 (25%) cases it was unmethylated. Whereas in only 2 out of 28 (7%) cases adjacent normal tissue showed methylated SFRP1 gene. Thus a significant difference in methylation status was present between tumor and non-tumor tissue. Methylated in relation to clinical stage was noted in 63% cases of stage II and 86.2 % cases of stage III tumor. To ascertain the methylation status of CpG sites present within promoter region of SFRP1 gene, we performed Bisulfite Sequencing of the 126 bp DNA fragment of SFRP1 gene amplified by MS-PCR in representative cases. Bisulphite sequencing showed methylated Cytosine nucleotide in the CpG sites. This 126 bp DNA sequence, in the cases showing methylated SFRP1 in MS-PCR, contained 22 CpG cites within which major no of the Cytosine were methylated.

SFRP1 promoter methylation associated with lymph node metastasis- Promoter methylation status of SFRP1 gene was compared with patient's clinicopathological characteristics such as age, tumor location, gender, lymph node involvement, tumor stage, and tumor grade. Chi square test results showed that lymph node metastasis was significantly associated with methylation status of SFRP1 gene in the tumor. Lymph node involvement (pN1-3) was noted in 84.6% cases with methylated SFRP1. Location of tumor

was not associated with methylation status. A higher incidence of methylation was detected in patients over 60 years age, however this was not statistically significant.

Methylation of status of *IGFBP-3* promoter- Methylation status of *IGFBP-3* gene promoter was determined by MS-PCR results in tumor. In this study, 37 cases out of 56 (66.1%) cases were methylated and 19 (33.9%) cases were unmethylated. *IGFBP-3* promoter methylation found in 13/27 (48.1%) cases of stage II and 24/29 (82.8 %) cases of stage III in tumor tissue. Patients of >50 year age group having slightly higher methylation frequency compare to <50 year age group the methylation frequency was 67.9% and 64.3% respectively. We found 61.8% male patients and 72.7% female patients had methylated *IGFBP-3* promoter. Methylation analysis also done in adjacent normal tissue but no methylation found in normal tissue which suggests that *IGFBP-3* promoter methylation could be a signature of malignancy.

Association of Clinicopathological features with promoter methylation of *IGFBP-3*- To determine whether promoter methylation status of *IGFBP-3* gene is associated with patient's clinical characters such as age, gender, tumor location, lymph node invasion, tumor stage, and histological grade, we performed Chi squire test. We found lymph node metastasis (p=0.006) was significantly associated with methylation of status of *IGFBP-3* gene promoter. Patients with positive lymph node (LN) metastasis (pN1-3) had 82.8% cases were methylated whereas only 48.1% cases were methylated with negative lymph node metastasis (pN0). On the basis of tumor site, 31 (75%) cases had methylated promoter out of 41 case of colon tumor and 6 (40%) cases of rectum tumor were methylated out of total 15 rectum cases. The association of promoter methylation with tumor site was not significant (P=0.013). In male 61.8 %, and female 72.7% tumor were having hypermethylated IGFBP-3 gene promoter.

Further, studies of promoter hypermethylation of the tumor suppressor genes such as SFRP1 and IGFBP3 also analysed and try to find associations on clinicopathological characters and the survival period of CRC patients. SFRP1 is an inhibitor of WNT signalling pathway, and transcriptional silencing leads to the WNT signalling activation promoting the tumorigenesis. In our study we found SFRP1 gene methylations in n 75% CRC cases, and is associated with lymph node metastasis. This clearly explains that

suppression of this gene makes the tumour aggressive and evade the neighbouring tissue. The IGFBP3 promoter hypermethylation found in this study is 66% in the CRC. SFRP1/IGFBP3 promoter methylations were significantly associated with poor survival and lymph-node metastasis, but in the later case it was found to be stage II CRC.

]To the best of our knowledge, no study has been examined the relationship between methylation of the *IGFBP-3, SFRP1 and CIMP marker* gene promoter with survival of patients in stage II and III of Colorectal cancer cases in India.