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Abstract Topic: - Molecular effects of genetic variation

Abstract Title: - Exploring contribution of genetic polymorphism in metabolic reprogramming in breast cancer.

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Aims: - To evaluate the effect of single nucleotide polymorphism in the FH (Fumarate hydratase) gene associated with breast cancer.

Methods: - 1.1 Study population: The study subjects were recruited from Max Super Specialty Hospital, Bathinda.

1.2 Selection and genotyping of SNPs: Blood sample was obtained from the confirmed patients for genomic DNA extraction. SNP selection was carried out by PCR amplification followed by Sanger sequencing. Individual SNP observed in most of the patients were selected for functional validation.

1.3 Bioinformatic analysis: Comprehensive analysis of the selected SNP was performed through various bioinformatic databases such as RegulomeDB, RNAfold, and PROMO.

Results: - rs1414493 is one SNP identified in the intronic region of the FH gene with no known function in breast cancer. rs1414493 represents a regulomeDB rank and score of 1f and 0.55324, respectively. The lower score indicates the variant is likely to affect the transcription factor binding and and linked to the expression of the target gene (FH). The variant is subjected to further analysis through RNAfold to check their impact on mRNA structure. RNAfold analysis revealed alteration in minimum free energy (MFE) and mRNA structure of wild-type and mutant-type. There were obvious changes in the structure of mRNA. An increase in loop size on the lower side of the mRNA is observed. A new loop was also introduced to the lower side of the mRNA. ΔG was found to be decreased from -194.80 to -196.20 (kcal/mol). This signifies the SNP as stabilizing for mRNA structure. The impact of variants on transcription factor binding sites on DNA sequence was evaluated through PROMO, which is a virtual library for the analysis of putative transcription binding factors. Both wild-type and mutated DNA sequences of SNP were used as input parameters for PROMO. The addition or removal of transcription factor binding sites (TFBS), as well as changes in the arrangement of these sites on DNA sequence, has been reported for the selected SNP. Apart from the rearrangement, there is a loss of TFBS for GR-beta in the mutant type. Simultaneously, a new TFBS for GATA1 is introduced into the mutant type. This addition and removal may lead to alteration in the transcription of the FH gene, subsequently resulting in faulty protein. Gene expression analysis of

GATA1 transcription factor and FH through the Gene expression omnibus (GEO) database manifested a decreased expression level of FH associated with the increased GATA1 expression in breast cancer patients. Pearson's pairwise correlation plot also confirmed a negative correlation between FH and GATA1. We then performed a Kaplan-Meier plotter to check the prognostic value of GATA1 in breast cancer. Overall survival (OS) is selected as a terminal event for the pooled Kaplan-Meier survival analysis. GATA1 showed a positive effect in the survival analysis.

Conclusions: - Since FH is considered a tumor suppressor gene, any mutation or alteration in the gene may contribute to the development of cancer. In our study, we found a new TF site of GATA1 at the SNP rs1414493 position, which may likely repress the transcription and produce a faulty protein. Alteration in the FH gene results in a defective oxidative phosphorylation (OXPHOS), which then causes a metabolic shift from OXPHOS to glycolysis (Warburg effect). This dual metabolic nature is a significant hallmark of cancer which enables the indefinite proliferation of cancer cells. Hence FH and GATA1 can be used as independent prognostic factors for breast cancer. The current state of knowledge about FH in the context of human cancer is still limited. Despite the distinctive role of FH in the development and progression of breast cancers, the integrated functions and prognostic values of FH in breast cancer are largely unexplored. Moreover, the role of GATA1 in breast cancer also needs to be investigated profoundly to link their association with the metabolic properties of cancer cells.

Keywords: - rs1414493 is one SNP identified in the intronic region of the FH gene with no known function in breast cancer. rs1414493 represents a regulomeDB rank and score of 1f and 0.55324, respectively. The lower score indicates the variant is likely to affect the transcription factor binding and and linked to the expression of the target gene (FH). The variant is subjected to further analysis through RNAfold to check their impact on mRNA structure. RNAfold analysis revealed alteration in minimum free energy (MFE) and mRNA structure of wild-type and mutant-type. There were obvious changes in the structure of mRNA. An increase in loop size on the lower side of the mRNA is observed. A new loop was also introduced to the lower side of the mRNA. ΔG was found to be decreased from -194.80 to -196.20 (kcal/mol). This signifies the SNP as stabilizing for mRNA structure. The impact of variants on transcription factor binding sites on DNA sequence was evaluated through PROMO, which is a virtual library for the analysis of putative transcription binding factors. Both wild-type and mutated DNA sequences of SNP were used as input parameters for PROMO. The addition or removal of transcription factor binding sites (TFBS), as well as changes in the arrangement of these sites on DNA sequence, has been reported for the selected SNP. Apart from the rearrangement, there is a loss of TFBS for GR-beta in the mutant type. Simultaneously, a new TFBS for GATA1 is introduced into the mutant type. This addition and removal may lead to alteration in the transcription of the FH gene, subsequently resulting in faulty protein. Gene expression analysis of GATA1 transcription factor and FH through the Gene expression omnibus (GEO) database manifested a decreased expression level of FH associated with the increased GATA1 expression in breast cancer patients. Pearson's pairwise correlation plot also confirmed a negative correlation between FH and GATA1. We then performed a Kaplan-Meier plotter to check the prognostic value of GATA1 in breast cancer. Overall survival (OS) is selected as a terminal event for the pooled Kaplan-Meier survival analysis. GATA1 showed a positive effect in the survival analysis.