FAT10 HAPLOTYPES AS A POTENTIAL BIOMARKER FOR CANCER

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1. Introduction

Cancer is the second leading cause of death today [1], accounting for nearly 1 in 6 deaths worldwide. Despite this, diagnosis and treatment models for cancer are limited and as such, new methods to identify and treat susceptible patients are required urgently. HLA-F-adjacent transcript 10 (FAT10) is an oncogene that is strongly implicated in the development of inflammation-associated cancers [2], notably the interaction between FAT10 and MAD2 was shown by in vitro and in vivo studies to promote malignancy [3-4]. Previous research on this highly polymorphic gene has identified 2 haplotypes – the reference haplotype, which is found in both cancer patients and healthy individuals, as well as an additional haplotype that occurs at higher frequency in cancer patients and is associated with higher odds of cancer. In this study, it was hypothesised that the cancer-associated FAT10 haplotype can better promote tumorigenicity compared to the reference haplotype and could thereby serve as a useful biomarker for individuals at risk of aggressive inflammation associated cancers.

2. Experimental Methods

In this study, HCT116 cell lines which stably overexpressed the 2 different FAT10 haplotypes (Ref. and Cancer haplotypes) were cultivated in McCoy's 5A Medium with 5% Fetal Bovine Serum (FBS) and incubated at 37°C with 5% CO2. As a negative control, HCT116 cells with an empty vector (VC) were used. To assess the ability of the cancer-associated haplotype in modulating various hallmarks of cancers, cell proliferation, apoptosis and soft-agar colony formation assays were conducted. Prior to analyses, HCT cells were sent for complementary DNA (cDNA) microarray profiling. After which, microarray analysis was performed using Partek® Genomics Suite® Software to determine differentially expressed genes between the 2 different FAT10 Haplotypes. Subsequently, pathway analyses were used to classify differentially expressed genes between the 2 FAT10 Haplotypes.

3. Results

Figure 1 shows the results obtained from the various assays. As shown in Fig. 1a, the cancer haplotype had the lowest doubling time amongst the 3 cell lines (18.2h), which was also significantly lower (p=0.044) when compared to the VC. This suggests that the cancer-associated haplotype promotes faster growth in cells. Furthermore, the cancer haplotype had significantly lower (p=0.0308) apoptotic cells (11.0%) upon CPT challenge relative to the reference haplotype (Fig. 1b), suggesting that the cancer haplotype best enhances resistance to cell death in cancer cells, one of the hallmarks of cancer. Additionally, the cancer haplotype conferred that greatest number of colonies (62 colonies) (Fig. 1c), and hence displayed significantly higher (p=0.0410) anchorage-independent growth relative to the

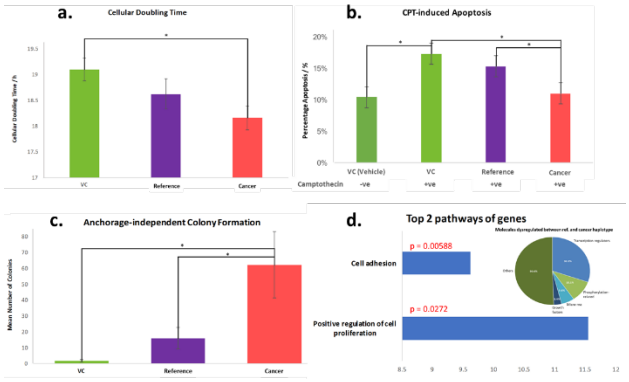

Figure 1 - Results obtained from various assays and analyses

reference. Finally, through analysis of microarray data, 61 genes were found to be differentially expressed between the 2 haplotypes. Of the 61 dysregulated genes, 30.3% of molecules were found to be transcription regulators and 10.1% phosphorylation-related (Figure 1d) through pathway analysis. Furthermore, the top 2 pathways these genes are associated with were found to be cell adhesion and proliferation, which play key roles in cancer epithelial mesenchymal transition and tumorigenesis. Hence, the dysregulation of these genes could be responsible for the observed differences.

4. Conclusion

This study had embarked on the functional characterisation of 2 FAT10 Haplotypes in cancer cells to establish its potential in promoting tumourigenesis. Through the various characterisation assays, the cancer haplotype was proven to enhance certain hallmarks of cancer compared to VC and reference haplotypes, namely sustained proliferation, resistance to apoptosis and anchorage-independent growth. Genes differentially expressed by the various FAT10 haplotypes were also uncovered and their functions analysed. It is believed that the modulation of certain molecules could have contributed to the increased tumourigenic potential of the cancer haplotype. Hence, our study sets precedence to future work and the full understanding of the potential of FAT10 haplotypes as biomarkers for cancer.

5. References

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