ABSTRACT

Colorectal cancer (CRC) is a significant health burden maintaining its position as the third most diagnosed cancer in men and women worldwide. Despite improvements in treatments for CRC, mortality rates still remain high. Genetic instability and epigenetic deregulation of gene expression are instigators of CRC development, resulting in genotype differences which herald treatment response variability and unpredictability. Over the past decade and a half, microRNAs (miRNA) have emerged as key contributors to the perturbed proteome in cancer cells, including CRC. MiRNAs are small non-coding RNA molecules (consisting of approximately 22 nucleotides) targeted to specific mRNAs through various target recognition mechanisms to repress protein translation or to induce mRNA degradation. Three miRNAs, miR-143, -145 and -133b, are most commonly downregulated in CRC and have been proposed as potential tumour suppressors. Although downregulation of these miRNAs in CRC is to a large extent unexplained, epigenetic silencing has been postulated as a causative regulatory mechanism. Potential epigenetic modulation of miRNA expression, by means of histone acetylation and DNA methylation, was assessed in this study by treating early (SW1116) and late stage (DLD1) CRC cells with the DNA demethylating agent, 5-aza-2'-deoxycytidine (5-Aza-2'C) and the histone deacetylase (HDAC) inhibitor, Trichostatin A (TSA), respectively. Subsequently quantifying miRNA expression, using miRNA TaqMan® PCR assays for each of miR-143, -145 and -133b, revealed that while all of these miRNAs are susceptible to DNA demethylation in early and late stage CRC cells, the susceptibility to DNA demethylation is significantly pronounced in the late stage DLD1 cells. Conversely, histone acetylation moderately affected miRNA expression in early stage CRC, but with a marginal effect on the expression of miRNAs in late stage CRC cells. These associations have been argued to correlate with genotypic differences between the microsatellite stable (MSS) SW1116 cell line and the microsatellite instability (MSI) of the DLD1 cells. To further evaluate the role that these miRNAs play in CRC development, this study utilised in silico miRNA target prediction tools to identify potential miRNA gene target lists. Once generated, these

were strategically curated and filtered to allow for the election of suitable candidates for functional analysis. This approach yielded three candidates, KRAS, FZD7 and FBXW11/ß-TrCP as the most probable targets for miR-143, -145 and -133b, respectively, further supported by their inverse correlations to the associated miRNA expression in CRC. Proteomic expression of the predicted targets assessed pre- and post- transfection of HET-1A cells with anti-miR™ sequences of the associated miRNA revealed elevated protein expression with differential subcellular protein localization upon miRNA inhibition. Overall this study has provided further understanding of the contribution of epigenetics in regulation of putative tumour suppressor miRNAs in CRC. Additionally, KRAS targeting by miR-143 has been reaffirmed, while FZD7 and FBXW11/ß-TrCP expression analysis after anti-miR-145 and anti-miR-133b transfection, respectively, provides substantial evidence for their role as potential direct miRNA targets.

Keywords: *colorectal cancer (CRC), epigenetics, demethylation, histone acetylase, 5-aza-2'-deoxycytidine, Trichostatin-A, SW1116, DLD1, miR-143, miR-145, miR-133b, anti-miR, KRAS, FZD7, FBXW11/ß-TrCP*