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Impact of aberrant miRNA CpG methylation on colorectal cancer progression and metastasis

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DNA methylation aberrations have been demonstrated to contribute to the early steps of malignanttransformation (Kunej, 2012). Originally, the first-described epigenetic change linked with a range ofsolid tumors was the hypomethylation (m5C residues replaced by unmethylated C residues) (Feinberg,1983). The resultant aberrant hypomethylation of specific DNA repeat elements or genes andchromosomal instability is believed to contribute to specific cancers and increased tumor frequencyand malignancy. Aberrant DNA methylation in CRC is recognized as one of the key features of cancerpathogenesis. A primary example is an average reduction of 10-30% total methylation levels incolorectal cancer relative to normal tissues. Furthermore, like other sequences within the genome,alterations of methylation of microRNA (miRNA) genes can regulate the expression of miRNAs topromote cancer development (Han, 2007; Lujambio, 2008). The aberrant DNA methylation pattern andmethylation-associated regulation of miRNAs were also observed in colorectal cancer (Han, 2007;Lujambio, 2008; Bandres, 2009).In this project, we identified the aberrant DNA methylation profile occurring in colorectal cancer,utilizing the Illumina HumanMethylation450 microarray. A total of 25342 differentially methylated CpGsites (DMC) were identified. The results showed significantly enriched CpG islands and shores to behypermethylated in promoter regions, whereas open sea regions were mostly hypomethylated in CRCtissues. These results suggest that DNA hypormethylation in open sea regions is a common feature ofCRC. Bioinformatics analysis showed that the most significantly methylated genes are not onlyenriched for cancer pathways such as PI3K-Akt signaling pathway, but also for pathways related toECM-receptor interactions and focal adhesion, which may directly or indirectly mediate cell activities,such as adhesion, migration, proliferation, and apoptosis in CRC.In our analysis, we were especially interested in the miRNA genes due to the track record of miRresearchin our department. Since in previous studies of our department the CRC metastasis-specificmiRNA expression profiles were investigated and found to play critical roles in the key steps ofmetastatic processes of CRC (Mudduluru, 2015), we focused on miRNA aberrant methylation profilesthat may be responsible for disturbed miRNA expression during metastasis in this study. 122 miRNAgenes were differentially methylated in tumors compared to normal tissues. From these, 16 genes ofmiRNAs were aberrantly hypermethylated and 108 hypomethylated. By integrating miRNAsexpression level in previous data at resected metastasis with gene methylation profile, ten miRNAs,comprising miR-34b, miR-34c, miR-329, miR-379, miR-154, miR-376b, miR-598, miR-654, miR-19aand -19b, were found to be significantly changed in methylation within their genes in the tumor tissuesas compared to normal colon tissues, which correlated with a significant change of expression in CRCmetastasis. Most of these genes showed methylation changes within open sea regions. Among thesemiRNAs, the miR-654 gene locus was hypomethylated in an open sea region in primary CRC tumorandcorresponding metastasis. Furthermore, data from MethHC showed that the degree ofmethylation across gene promoter regions as well as CpG islands, shelves, shores and expression ofmiR-654 was positively correlated. An MSP assay after 5-aza-CdR-induced demethylation indicatedthat CpG hypomethylation in open sea regions may be related to the reduced expression of miR-654.It elucidates our previous findings in metastatic lesions and may reveal the mechanism underlying thedownregulated expression of miR-654 in metastatic colon cancer cells. To date, there have been noprevious reports of an effect of CpG open sea methylation on miRNA expression in colorectal cancercells. Subsequently, the forced overexpression of miR-654 significantly suppressed cell proliferation,migration and invasion in at least 2 different CRC cell lines, suggesting that miR-654 triggered aninhibitory effect on the initial steps of metastasis. Bioinformatics analysis suggested that miR-654targets multiple cancer pathways that

promote cell proliferation and metastasis.In conclusion, this research showed the overlap between genome-wide mapping of differentiallymethylated CpG sites/ differentially methylated regions and miRNA landscape changes during theprogression of CRC, indicating that epigenetic changes may account for the initiation and progressionof CRC. Among the overlapping set of microRNAs, open sea hypomethylation of the miR-654 codinggene resulted in reduced expression of miR-654. All these support a suppressor function of initialsteps of metastasis of this miRNA and its subsequent repression of cancer-related signaling pathways.Our results highlight a more comprehensive understanding of DNA methylation alterations affectingmiRNA expression, and provide insights for potential new targets in the treatment of CRC.

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