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Thesis abstract

Folate and vitamin D: The role of nutritional status and nutrigenetics in predicting levels of extracellular microRNA and circulation DNA methylation

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MicroRNA (miRNA) in systemic circulation are proposed as potential biomarkers for disease diagnosis and prognosis. However, miRNA profiles may also be modulated by other exposures such as nutritional status, and this may have consequences for use of miRNA as biomarkers, particularly in diseases for which diet is a modifiable determinant. Furthermore, little is known about the interactions that exist between these relationships and underlying variance in genes related to the processing of nutrients that may influence these relationships, or how these miRNA interact with other modifiers of gene expression, such as DNA methylation.

This thesis focuses on folate and vitamin D, two key micronutrients known to have the potential to influence gene expression. The data presented here investigate the relationships between these micronutrients and related nutrigenetics in predicting levels of extracellular miRNA and circulating DNA methylation status. The studies presented here were designed to capitalise on the availability of two well-characterised human cohorts: a case-control cohort of adenomatous polyp patients and healthy controls (n=263), and an elderly cross-sectional cohort (n=649). These are appropriate cohorts in which to investigate these relationships, as systemic circulating miRNA have been proposed as biomarkers for adenomatous polyps and colorectal cancer (CRC), diseases with known dietary modifiers of risk (including folate and vitamin D) which accumulate over a lifetime of exposures. Four candidate miRNA (let-7a, miR-15a, miR-21 and miR-155) were selected due to a combination of factors: each has known oncogenic or tumour-suppressor properties and each had existing evidence to suggest potential regulation by nutritional factors.

The first results chapter (Chapter 2) presents novel observations on the levels of systemic circulating levels of let-7a, miR-15a and miR-155 in adenomatous polyp cases relative to controls. Furthermore, by adding a sex specific level of analysis, it adds to the body of knowledge surrounding these miRNA and miR-21, which is currently proposed as a biomarker for adenomatous polyps. Novel data on the correlations between blood levels of folate and related micronutrients and the candidate miRNA are presented, with key findings including a positive correlation between red blood cell folate levels and all candidate miRNA, regardless of their tumour-suppressor or oncogenic properties. Stepwise regression analyses investigating the

correlations between systemic circulating miRNA levels and multiple dietary intakes, including vitamin D, are also presented.

Chapter 3 builds upon these results by incorporating common folate and vitamin D related genetic polymorphisms into the analyses. The relationships between these polymorphisms, systemic circulating miRNA levels, and risk for adenomatous polyps were assessed, as well as interactions with nutrient status. Statistically significant relationships were identified between multiple polymorphisms and risk for adenomatous polyps, and miRNA levels, as well as potential interactions between folate status and genotype in predicting miRNA levels. These are the first reported observations of the potential relationships and interactions between miRNA profiles and nutrigenetic variance.

As the human cohorts used can only demonstrate correlation and not causation, Chapter 4 contains data obtained from cell culture models. Three CRC cell lines were used to demonstrate that miRNA are differentially expressed intracellularly and extracellularly under folate excess or deficient conditions, and following stimulation with the active vitamin D metabolite. Treatment with a DNA demethylating agent was also used to demonstrate that some of these processes are dependent on DNA methylation.

The relationships between vitamin D and DNA methylation were further investigated in Chapter 5. A sub-cohort was used to conduct a pilot study investigating the relationships between vitamin D status, methyl donor-related micronutrients and DNA methylation in genes of vitamin D metabolism. The relationship between methylation status in this pathway and the systemic circulating levels of the candidate miRNA were also assessed, and provides new information demonstrating the potential complexity of the complementary pathways for the regulation of cellular processes and pathways.

Together, the data in this thesis constitute a significant contribution to the body of knowledge surrounding the extracellular levels of miRNA, and how this may relate to vitamin D and folate status, related polymorphisms, DNA methylation, and intracellular miRNA expression levels. Relationships were identified between folate status, nutrient intake and systemic circulating levels of multiple candidate miRNA. Relationships identified between polymorphisms in related genes and systemic circulating miRNA levels support these observations, and these observations may link dietary factors to modified risk for disease.

This thesis expands our understanding of how nutrition and nutrigenetics can interact to modify nutrigenomics and disease risk. The data presented here for the candidate miRNA and two key nutrients, provide an impetus to investigate these relationships for other nutrients and miRNA, particularly those known to modify disease risk. These results have implications for the use of systemic circulating miRNA as biomarkers, and may also have implications for the future of personalised nutrition and personalised medicine.

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