

Calorie Restriction (CR)

Calorie Restriction

Histone deacetylation

Sirt1

DNA methylation

Molecular Basis of the Effects of CR on Ageing

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The Investigation

It is well established that CR in rodents, and in other model organisms, increases lifespan and reduces the incidence of and/or delays the onset of ageing-related diseases. The study is aimed towards understanding this effect at the molecular level, with a focus on the role of the protein Sirt1, whose increased expression appears to be pivotal in the beneficial effect of CR on ageing. Our hypothesis is that Sirt1, which has histone deacetylase activity, mediates these effects through changes in histone acetylation status (Fig. 1), which, in turn, affect patterns of DNA methylation (Fig. 2). DNA methylation is altered with ageing and aberrant DNA methylation can result in appropriate gene expression or silencing so may be causal in the ageing process.

Objectives

1. Establish proof of principle that increasing the level of expression of Sirt1 in the human colonic cell line SW480 can alter global and site-specific DNA methylation.
2. Establish proof of principle that increasing the level of expression of Sirt1 in a model system (EGFP reporter gene controlled by the CMV promoter in HEK293 cells) replicates reported effects of the manipulation of histone acetylation status on reporter gene methylation.
3. Determine if manipulation of Sirt 1 expression in the SW480 and HEK293 cell lines alters histone acetylation.

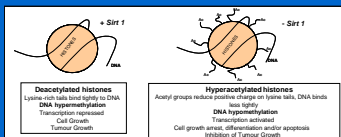


Figure 1. The relationship between histone acetylation status, DNA methylation and gene transcription and proposed effects of Sirt1

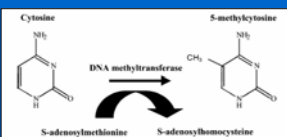


Figure 2. DNA methylation - catalytic conversion of cytosine to 5-methylcytosine by DNA methyltransferase

Methodology

Sirt1 expression will be increased in cell lines, to mimic the effects of CR, by overexpression of a human Sirt1 transgene. Effects on global methylation and on site-specific methylation of CpG islands in the *ER*, *IGF2* and *c-MYC* genes, and on the EGFP reporter gene, will be determined. DNA methylation will be measured by bisulphite sequencing, involving the conversion of all unmethylated cytosine bases to uracils by treatment with sodium bisulphite followed by PCR amplification and sequencing (Fig 3).

Collaborator

Professor John Mathers, Newcastle University

Potential Benefits

For older people and society

Diet is an attractive target for healthcare strategies aimed at delaying the ageing process. It is important that we gain a better understanding of the impact of nutrition on the ageing process to underpin advice on maximising health through diet. At present, CR appears the most effective dietary strategy for delaying the ageing process in model organisms, but it is currently unknown to what extent this is an effective measure in humans, nor is it effective if introduced later in life.



The findings of the project will contribute to improved understanding of the molecular basis of the beneficial effect of CR on ageing to inform dietary advice and potentially guide the discovery of therapeutic or alternative dietary interventions to delay the ageing process. The findings will also indicate if DNA methylation might be an informative biomarker of a positive effect of CR and thus provide a measure of efficacy in older people to guide research in this group. The project will indicate if resources should be directed, in the future, towards exploring the importance of changes in DNA methylation in the beneficial effects of CR on ageing.

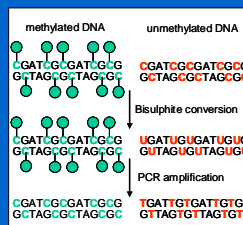


Figure 3. Measurement of DNA methylation by bisulphite sequencing. Unmethylated, but not methylated, cytosines are converted to thymines.

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