Caffeine exposure and folate deficiency *in vitro* can influence chromosomal translocation events associated with childhood leukaemia

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Background

Childhood leukaemia incidence is increasing, with various environmental factors associated with risk. Some leukaemia-associated genetic abnormalities, such as chromosomal translocations, have been retrospectively detected at birth, suggesting they may originate *in utero,* but causes are still unknown.

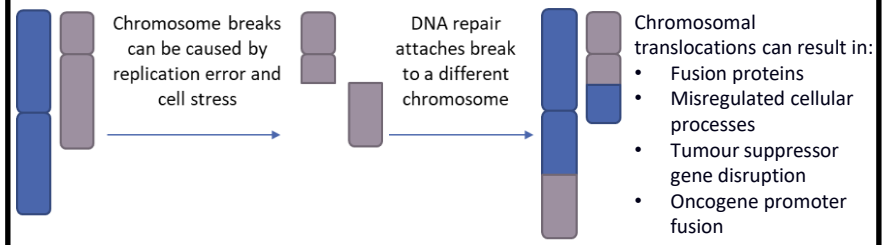

Figure 1. Overview of reciprocal chromosomal translocation induction and potential consequences.

Caffeine

- Half life is increased during pregnancy.
- Can cross the placenta, testis and into breast milk.
- Shown to affect DNA repair and cell cycle checkpoint pathways.

Folate deficiency

- Required for nucleotide biosynthesis & methyl donors.
- Deficiencies can lead to DNA breaks and altered DNA methylation.

Aim: to investigate if environmental exposures may induce childhood leukaemia associated translocations.

Methods

Leukaemic NALM6 cells exposed to physiological levels of caffeine and folate (3 replicates). Reverse Transcription PCR assays developed for leukaemia translocations RUNX1-RUNX1T1 and TCF3-PBX1.

Caffeine protocol

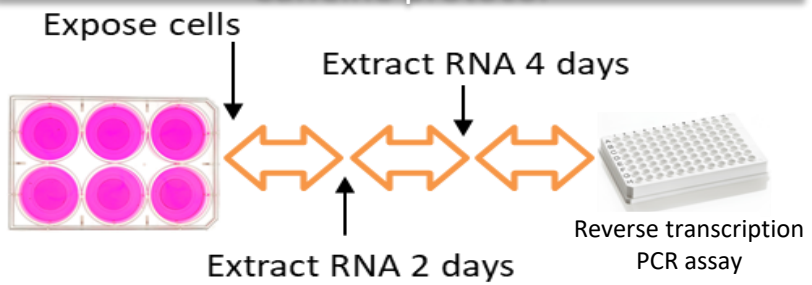

Figure 2. Experimental design for identifying translocations in NALM6 cells exposed to caffeine.

Folate deficiency protocol

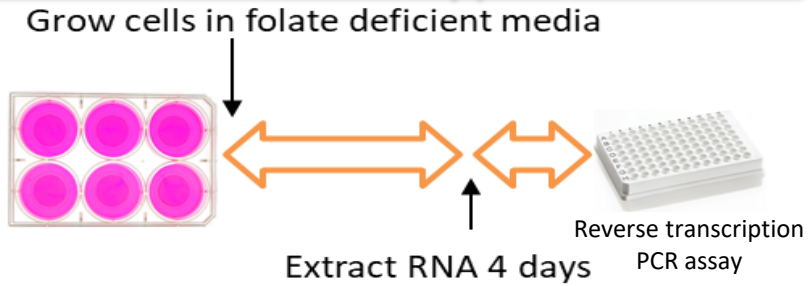

Figure 3. Experimental design for identifying translocations in NALM6 cells exposed to various folate concentrations.

We would like to thank Northumbria University and Children with Cancer UK for funding this project.

Results

Table 1. Chromosomal translocation events in NALM6 cells in response to folate deficiency or caffeine exposure.

		RUNX1-RUNX1T1		TCF3-PBX1	
Exposure concentration	Physiological range	2 d	4 d	2 d	4 d
DMSO Control	Control				
80uM Caffeine	Very, very high				1
40uM Caffeine	Very high				
20uM Caffeine	High				
10uM Caffeine	Medium			1	
2uM Caffeine	Low				
2000nM Folic Acid	Normal TC media				
200nM Folic Acid	Very high				
100nM Folic Acid	High		1		
50nM Folic Acid	High end normal range				
10nM Folic Acid	Low end normal range				1
5nM Folic Acid	Low				
1nM Folic Acid	Depleted				1
0.1nM Folic Acid	Deficient				

Summary and future work

- Preliminary results indicate medium and very high caffeine exposure and deficient and lower physiological levels of folate induce translocations.
- Future work will validate an optimised cell model in multiple cell lines and confirm positive translocations with FISH assays.