Circulating free DNA: a 'liquid biopsy' for the early detection of cancer?

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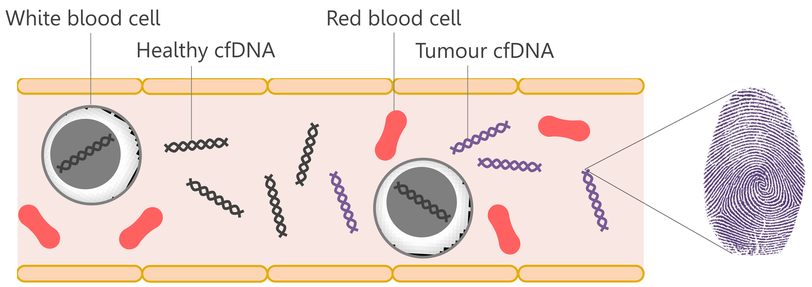
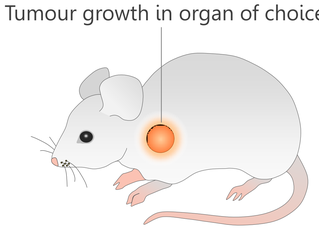
MRC Medical Research Council

Circulating free DNA



- Blood contains tiny amounts of free-floating DNA, called circulating free DNA (cfDNA)
- It is thought to leak into the blood from normal cells when they die
- In patients with cancer, DNA leaks into the blood from dying tumour cells
- Cancer is a genetic disease – thus, tumours release their genetic 'fingerprints' into the blood

Mouse models



- Early-stage cancers often go undetected, so it is difficult to study cfDNA in these patients
- Mouse cancer models can mimic the human disease and could help determine **how early** cfDNA reveals signs of disease

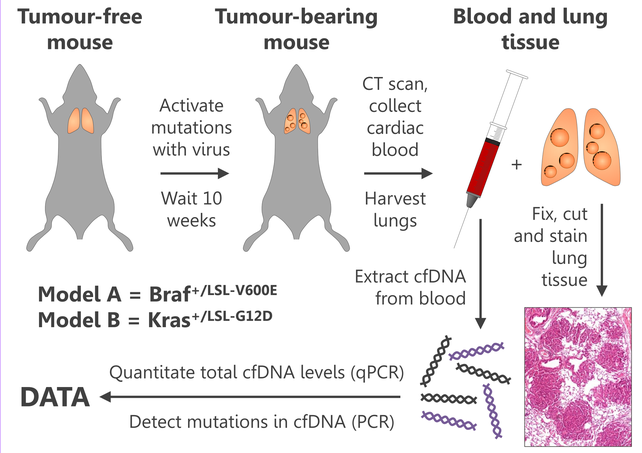
Research question

Can we use cfDNA as a non-invasive 'liquid biopsy' to detect cancers before other tests?

Study aim

To determine whether cfDNA can be isolated and quantitated from small volumes of blood in two well-characterised mouse models of lung cancer

Methods



Conclusions

- This study has detected higher levels of cfDNA in the blood of mice with pre-cancerous lung lesions, and has identified the lesion-causing mutations in the cfDNA
- An appropriate next step would be to allow the lung lesions to develop over a longer period of time, to allow them to become cancerous
- Collection of blood over a series of time points could determine if the cancerous lesions leak more mutated DNA as they grow larger

Acknowledgements

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- Susan Giblett for her kind technical support
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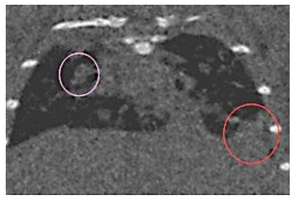
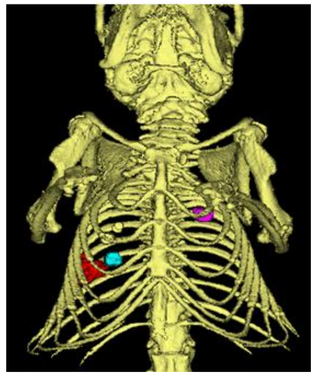
Results

1) Pre-cancerous lung lesions were identified at 10 weeks by CT scanning

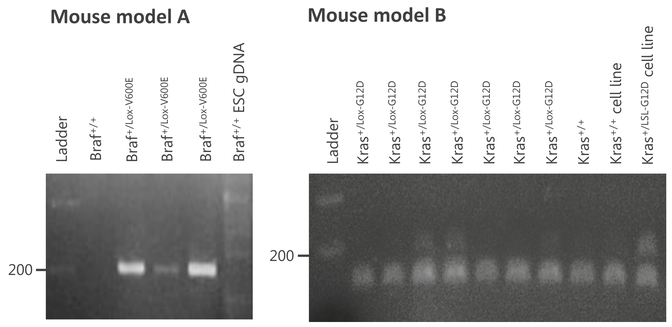
Right: circles show pre-cancerous lung lesions after 10 weeks by CT scanning.



Right: many images produced by the CT scanner can be combined into a 3D image. The coloured spots indicate pre-cancerous lung lesions.

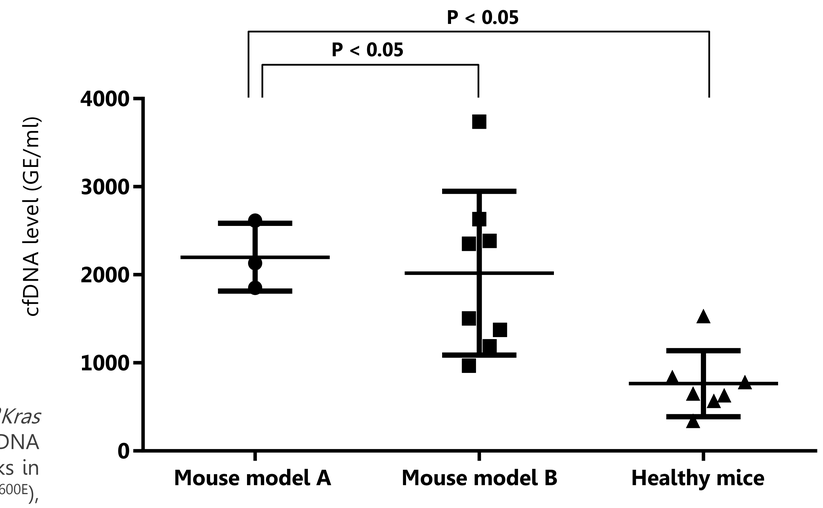


2) The tumour-causing mutations were identified in cfDNA of both models at 10 weeks, but not in cfDNA of healthy mice



Left: the mutant ^{V600E}*Braf* and ^{G12D}*Kras* alleles can be detected in cfDNA isolated from plasma after 10 weeks in some mice. In model A (Braf$^{+/LSL\text{-}V600E}$), PCR primers amplify a ~ 200 bp region specific to the mutant allele, but not the WT. WT mouse embryonic stem cell (ESC) DNA did not amplify. NTC, no template control. In model B (Kras$^{+/LSL\text{-}G12D}$), PCR primers amplify ~ 140 bp and ~ 180 bp regions corresponding to the WT and mutant alleles, respectively. The mouse genotype is shown above each lane.

3) cfDNA levels were elevated in both models at 10 weeks, compared to healthy mice



Above: After 10 weeks, model A mice (Braf$^{+/Lox\text{-}V600E}$; n = 3) had an average cfDNA concentration of 2200 genome equivalents (GE)/ml blood, and model B mice (Kras$^{+/Lox\text{-}G12D}$; n = 8) had an average cfDNA concentration of 1970 GE/ml blood. Healthy controls (WT; n = 7) had an average cfDNA concentration of 760 GE/ml blood. Lines represent the mean +/- standard deviation. GE = genome equivalent = 6.6 pg DNA/cell.