Red Meat and Colorectal Cancer – Molecular Links and Future Prospects

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Mutations in Colorectal Cancer

- Mutations in *APC*, *p53* and *Kras* genes could be caused by dietary mutagens….
- …. But, out of the thousands of possible chemicals in diet what might be responsible?
- The exquisite sensitivity of colorectal cancer to induced (‘somatic’) mutations is known through studies of genetic predisposition – in cases where all cells already have one mutation (such as HNPCC).
A digression into N-nitrosation

N-Nitrosation is a simple chemical reaction that can have profound biological consequences.

\[
\begin{align*}
\text{R'}NH & \xrightarrow{\text{ON-X}} \text{R''NNO} + \text{HX} \\
\end{align*}
\]

*Usually biologically inactive* (active ?) Toxic, mutagenic, carcinogenic

Dose response in faecal ATNC levels in 9 volunteers with change in red meat levels (Bingham, Hughes, Cross 2002)

Faecal ATNC

G red meat per day (Diet NOC content 13 ug/day Silvester et al 1997)
Change in faecal ATNC levels: high red meat and high protein vegetarian diet compared with low red meat diet 65 v 150 g protein (Cross, Bingham et al Cancer Research 2003)

Change in faecal ATNC on changing from a low meat to a high red or white meat diet (Bingham, Cross, Hughes 2002)
The effect of dietary iron and haem on faecal ATNC levels (Cross, Bingham et al 2003)

Haemoglobin

Myoglobin
N-Nitrosation appears to be important…..

….. But, what is being nitrosated?

Nitrosation of bile acids

➢ Reflux of bile acid conjugates into gastric juice is associated with increased risk of cancer

Induces gastric cancer and intestinal metaplasia following dosing by intubation (Charnley et al 1985)
The conversion of glycine to DNA-damaging agents through nitrosation

An antibody was developed which recognised this chemically modified DNA base with great sensitivity and specificity.

Isolation of exfoliated colon cells

Hematoxylin stained exfoliated colonocytes. Single cells (A) as well as clusters (C) can be clearly identified.

Bandaletova, Bailey, Bingham, Loktionov 2002 APMIS 110 239-46
Davies, Freeman, Morris, Bingham, Dilworth, Scott, Laskey, Miller, Coleman 2002 Lancet 359 1917-9
**Immunochemical localisation of O\(^6\)CMdG in exfoliated colonic cells**

HT 29 cells incubated with diazoacetate

Rat small intestine

Untreated colonic exfoliated cells isolated from humans eating normal diet

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### Increase in fecal ATNC on change from a vegetarian to a high (420g) red meat diet (Lewin Shuker, Bingham et al Cancer Research 2006)

![Graph showing increase in fecal ATNC](image_url)
Increase in O6-carboxymethylguanine adducts in exfoliated colonic cells on changing from a vegetarian to a high (420 g red meat diet) (Lewin Shuker, Bingham et al Cancer Research 2006)

Correlation between fecal ATNC and percent cells staining positive for O6-carboxymethylguanine (Lewin Shuker, Bingham et al Cancer Research 2006)

\[ r = 0.56 \quad p < 0.001 \]
Quantitation of O\textsuperscript{6}CMdG

O\textsuperscript{6}CMdG in human blood DNA


P53-containing plasmid

Srikanth Ponnada, Yao-Zhong Xu, Phil Burns (Leeds)
Mutagenicity of $O^6$CMdG

**Control DNA**

$25-O^6$-CMdG DNA

Srikanth Ponnada and Phil Burns, unpublished results

**KDA**

- GC to AT
- GC to TA
- AT to GC
- AT to TA
- AT to GG

**MNU**

- GC to AT
- GC to TA
- AT to GC
- AT to TA
- AT to GG

**Stomach**

**Colon**
Nitrosation of glycine (or glycine-containing substrates) is probably the source of O⁶CMdG, as well as (some) O⁶MedG, in human DNA.

Lack of repair of O⁶CMdG makes it a potentially very useful biomarker, but it is also contributing to mutagenicity.

On-going prospective studies will tell us how good O⁶CMdG is as a marker of colorectal cancer risk.

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**European Prospective Investigation on Diet and Cancer**

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SEQUENCE LEADING TO NEOPLASIA

<table>
<thead>
<tr>
<th>Initiation</th>
<th>Promotion</th>
<th>Conversion</th>
<th>Progression</th>
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<tbody>
<tr>
<td>Viruses, radiation, chemicals</td>
<td>Procarcinogens</td>
<td>Direct carcinogens</td>
<td>Initiated cell</td>
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<td>Ultime carcinogens</td>
<td>Genetic change</td>
<td>Preneoplastic lesion</td>
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INTERVENTION STRATEGIES

- **Primary Prevention**
  - Avoid exposure
- **Chemoprotection**
  - Prevent formation of carcinogens
- **Early Diagnosis**
  - Block interactions with genome
  - Suppress growth
- **Secondary Prevention**
  - Early diagnosis
- **Therapy**
  - Surgery
  - Radiotherapy
  - Chemotherapy
- **Tertiary Prevention**
  - Prevent relapse & secondary tumours

Biomarkers of exposure and risk

Measures of exposure or early biological effect

Indicators of risk
Future prospects

- We currently have very good simple biomarkers for very effective interventions to reduce cardiovascular disease (BP, serum cholesterol, etc)
- We need similar biomarkers for common preventable cancers
- Markers of DNA damage such as O\textsuperscript{6}CMdG are promising leads

The source of the problem!