RNA Sequencing of Colorectal Cancer Tumour and Lymph Node Samples Characterises Differential Gene Expression in Old and Young Patients

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Introduction
Incidence of colorectal cancer (CRC) increases with age, with older patients having a worse prognosis than younger patients. The underlying molecular features which lead to these age-related differences are unclear, but possible reasons include immune senescence or cellular senescence. Characterisation of differences in gene expression in cancers of older and younger patients, particularly genes driving the immune response to cancer, will contribute to our understanding of the differences between early- and late-presenting tumours and will inform treatment strategies.

Methods
RNA was extracted from tumour cells and lymph nodes from three cohorts of patients with Rectal Cancer (n=47), Stage 2 CRC (n=72) and Stage 3 CRC (n=69). For the Stage 3 CRC cohort, RNA was extracted from the primary tumour, one normal lymph node and one metastatic lymph node. After RNA-sequencing, Differential Gene Expression Data was generated between older and younger patients in the Stage 3 cohort, with ‘old’ patients being one standard deviation (SD) above the median, and ‘young’ patients one SD below the median (median = 72 years). Results were validated with a second cohort of Stage 3 CRC samples (n=78).

Results
In primary tumour samples from the Stage 3 Cohort, there were 581 genes differentially expressed between older and younger patients at p-adj <0.05, and 159 genes differentially expressed in metastatic lymph nodes between the two groups.

Gene Expression and Gene Enrichment Results for Stage 3 Late-presenting CRC

<table>
<thead>
<tr>
<th>CRC Cohort</th>
<th>No. of DE genes at padj&lt;0.05</th>
<th>Top five DE genes</th>
<th>Downregulated gene sets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 3 Primary Tumours</td>
<td>581</td>
<td>1. PCSK9</td>
<td>Oxidative Phos. MYC V1&amp;V2 MTORC1 E2F UPR</td>
</tr>
<tr>
<td>Stage 3 Metastatic Lymph Nodes</td>
<td>159</td>
<td>1. ACAA2</td>
<td>E2F G2M MYC V1&amp;V2 Oxidative Phos. MTORC1</td>
</tr>
</tbody>
</table>

In older patients, we found downregulation of the Oxidative Phosphorylation, MYC V1 and MYC V2 gene sets and, interestingly, downregulation of Unfolded Protein Response (UPR) genes.
Under normal conditions, the UPR promotes apoptosis in conditions of prolonged cellular stress; downregulation of UPR genes may therefore enhance tumour survival by abrogating this response. Although downregulation of UPR genes may be a natural part of cellular senescence, this change might be exploited by late-presenting tumours.

Conclusions
Our results have shown that there are detectable differences in tumour and lymph node gene expression between older and younger patients; such findings may further enhance age-dependent personalised therapy. We have chosen to focus on age-related differential gene expression, but differences between other tumour phenotypes may also be investigated and are likely to further enhance our understanding of cancer biology.