Colorectal adenoma subtypes exhibit distinct molecular profiles

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Background

• Colorectal cancer (CRC): 2003 unique hypermethylated DMRs (Fig. 1) that are differential among histologies but not with NATs
• Around 85% of colorectal cancers are believed to originate from the malignant transformation of advanced precancerous lesions (APL).
• As methylation, mutations and copy-number alterations (CNAs) are well described in CRC

• Colorectal adenoma subtypes exhibit distinct molecular profiles

Methods

Whole Genome Bisulfite Sequencing (WGBS) 30x
Identification of Differentially Methylated Regions (DMRs)
Validation of DMRs
GO detection and somatic mutation analysis
GO and KEGG pathways analysis

Results - I

• 2003 unique hypermethylated DMRs (Fig. 1) that are differential among histologies but not with NATs
• DMRs are distributed along the entire genome with an enrichment in chromosome 7 (Fig. 2 and bar-plot in Fig. 4)

Results - II

• DMRs separate APL samples by histological subtype in both indentification and validation set (Fig.3).
• The CNA signals were mostly present in TA or VATVTA samples (Fig.4). The most frequent signals could be seen in chr7, 12, 19 and 20, while majority of CRC I exhibited CNA in chr 7, 8 and 20, indicating difference processes to be in place between APL and CRC I progression. The enrichment in number of events in chr7 can be significantly associated to enrichment in number of DMRs on the same chromosome (Fig.2 and Fig. 4).
• Somatic mutation analysis indicates differences between histological sub-types, underlined in different oncological pathways enriched (Fig. 5).
• KEGG pathway analysis revealed significative enrichment in categories directly or indirectly related to cancer (Fig. 6)

Results - III

• Colorectal adenoma histological subtypes show distinctly different methylation, CNA and mutational signals, between groups and compared to CRC I.

• Characterization APL subtypes could aid in developing early detection or cancer prevention tests.

Table 1: Main patient features

<table>
<thead>
<tr>
<th>Identification of DMRs</th>
<th>Validation of DMRs</th>
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<tbody>
<tr>
<td>APL paired with NAT</td>
<td>CRC stage I paired with NAT</td>
</tr>
<tr>
<td>Age median (Q1, Q3)</td>
<td>65 (51, 71)</td>
</tr>
<tr>
<td>Sex, n</td>
<td>187 (89)</td>
</tr>
<tr>
<td>Gender Percentages, n</td>
<td>139 (69)</td>
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<td>Stool Sample, n</td>
<td>252</td>
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</table>

Figure 1: DMR selection. UpSet plot visualizes the intersection among the different sets of significant hypermethylated regions. To enrich the histological subtype signals, only those regions that do not overlap with APL vs NAT results are taken into account (red circles).

Figure 2: Genomic Circular Visualization of Regions Distribution Along Genome. The density layer of the plot emphasizes an enrichment of DMRs on chromosome 7. The outermost layers reveal the top 30 genes that significantly contribute to the PCA showed in Figure 3.

Figure 3: PCA analysis of methylation data. A) PCA analysis depicting three largest components of variance in beta-values in SSL (n=14), TA (n=26) and VATVTA (n=34). B) Boxplot of contributions on first dimension

Figure 4: CNA analysis. Heatmap of number of CNA events divided per chromosome for each APL sub-type and CRC stage I. Bar-plot on the right indicates the DMR regions enrichment per chromosome. Chr7 has a significant correlation between CNA events and hypermethylated regions.

Figure 5: Oncological pathway enrichment. Enrichment of mutated genes in known oncogenic pathways indicates differences among sub-types but also between APL and CRC stage I.

Figure 6: KEGG enrichment analysis of the identified 2003 DMRs. Dot plot showing the KEGG analysis for the identified differentially methylated genes. Dots are color-coded from blue to red based on the adjusted p-value. The size of the dots is proportional to gene count.

Disclosure statement

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KK is employees of UniversalDx d.o.o., PFM and PCN are employees of Universal Diagnostics S.A.

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Bibliography