Comparison of KRAS, NRAS and PIK3CA mutational status in poorly differentiated clusters (PDC) and corresponding main tumor mass in colon cancer

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Poorly differentiated clusters (PDC)

New Criteria for Histologic Grading of Colorectal Cancer

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Clusters composed of ≥ 5 cancer cells and lacking a gland-like structure, in the stroma or at the invasive front of colorectal cancer (CRC)
Grading based on PDC counting in CRC

G1 (<5 PDC*)
G2 (5-10 PDC*)
G3 (≥ 10 PDC*)

*Maximum number in a x20 objective lens field

More reproducible than WHO grade
High grade associated with nodal metastases
Significant prognostic value independent from pTNM stage

PDC show epithelial-mesenchymal transition phenotype and may represent neoplastic cells with higher invasion properties.
Molecular alterations in CRC

KRAS/MAPK pathway

Deregulation in 55% CRC

Mutations of KRAS, BRAF and NRAS genes activate the pathway independently from ligand binding and give resistance to anti-EGFR therapy

KRAS mutations in 40% CRC
KRAS mutations significantly more frequent in PDC G3 CRC ($P = 0.0379$)

High frequency of KRAS mutations in exons 3/4 in PDC G3 CRC
To investigate the mutational status of KRAS, NRAS and PIK3CA in the PDC of a series of colonic carcinomas (CCs) known to have KRAS mutations

To compare the mutational status observed in PDC with that revealed in the corresponding main tumor
Materials and methods

25 consecutive FFPE CCs with mutated KRAS and > 10 PDC at the invasive front

Laser Micro-dissector Olympus CKX41
Materials and methods (I)

DNA PDC

KRAS/NRAS/PIK3CA mutational analysis with Mass array system

DNA main tumor
## Results

<table>
<thead>
<tr>
<th>KRAS mutation</th>
<th>Main tumor (n)</th>
<th>PDC (n)</th>
<th>NRAS/PIK3CA main tumor (n)</th>
<th>NRAS/PIK3CA PDC (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G12D</td>
<td>11</td>
<td>11</td>
<td>PIK3CA H1047R (1)</td>
<td>PIK3CA H1047R (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PIK3CA E542K (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PIK3CA E545Q (1)</td>
</tr>
<tr>
<td>G12A</td>
<td>4</td>
<td>3 (1 wt)</td>
<td>PIK3CA E545K (1)</td>
<td>PIK3CA E545K (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NRAS G12D (1)</td>
<td>NRAS G12D (1)</td>
</tr>
<tr>
<td>G12C</td>
<td>1</td>
<td>0 (1 wt)</td>
<td>wt</td>
<td>wt</td>
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<tr>
<td>G12S</td>
<td>1</td>
<td>1</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>G12R</td>
<td>1</td>
<td>1</td>
<td>PIK3CA C420R (1)</td>
<td>PIK3CA C420R (1)</td>
</tr>
<tr>
<td>G13D</td>
<td>1</td>
<td>1</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>G13C</td>
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<td>1</td>
<td>wt</td>
<td>wt</td>
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<td>wt</td>
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<tr>
<td>A59T</td>
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<td>wt</td>
<td>PIK3CA H1047Y (1)</td>
</tr>
<tr>
<td>A146T</td>
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<td>1</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>K117N</td>
<td>1</td>
<td>1</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>Total mutated</td>
<td>25</td>
<td>23</td>
<td>PIK3CA (3)/NRAS (1)</td>
<td>PIK3CA (6)/NRAS (1)</td>
</tr>
</tbody>
</table>
Results (I)

KRAS mutated CRC

- 80% with same KRAS/NRAS/PIK3CA mutations in main tumor and PDC
- 12% with KRAS wt in PDC
- 8% with PIK3CA wt in main tumor and mutated in PDC
Results (II)

All 3 cases with PIK3CA mutations in PDC but not in main tumor had nodal metastases and high pTNM stage
Conclusions

KRAS mutated CRC with high number of PDC have high frequency of rare KRAS (codons 59, 61, 117) and PIK3CA (E545Q, H1047Y, C420R) mutations: pathogenetic association?

PDC may show different molecular profile compared to main tumor

Heterogeneity in molecular profile of different areas of CRC may depend upon dissimilar histological aspect and differentiation
Future perspectives

Impact of molecular heterogeneity between PDC and main tumor on efficacy of anti-EGFR therapy?

• If metastases have the same mutational status as PDC and different from the main tumor, KRAS mutations should be better evaluated in PDC to predict response to anti-EGFR therapy.
Thank you for your attention