Effects of phosphatidylcholine-specific phospholipase C inhibition on tumour growth, metabolism, and HER2 expression in preclinical models of HER-2 overexpressing ovarian cancer

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Introduction - The discovery of an abnormal choline metabolism as a hallmark of cancer warrants investigations on the role of the activities of phosphatidylcholine (PtdCho) cycle enzymes as potential molecular indicators of tumor response and new targets for anticancer treatment [1,2]. The altered choline profiles detected by high resolution MRS in human epithelial ovarian cancer (EOC) cell lines compared with nontumoral counterparts were associated with 12-25x increase in choline kinase (ChoK) activity and 5-17x activation of phosphatidylcholine-specific phospholipase C (PtdCho-PLC) [3]. We focused our attention on biological and metabolic effects of in vivo passage on the human HER2-overexpressing SKOV3 cell line, which allowed selection of cells (SKOV3.ip) endowed with a more aggressive phenotype, enhanced HER2 expression and higher PtdCho-PLC activity [4]. These features were associated with a higher phosphocholine (PCho) level in SKOV3.ip cells compared with the parental cell line [4; Pisanu et al, manuscript in preparation]. Purpose of this work was to investigate the role of PtdCho-PLC inhibition as a possible new approach to target in vivo tumorigenicity of HER2-overexpressing EOC cells, using as a model xenografts of SKOV3.ip cells in immunodeficient mice.

Methods - Cells: SKOV3.ip cells were established from the in vivo passage HER2-overexpressing SKOV3 cell line, as described in ref. 4. High resolution MRS analyses were performed on cell and tissue extracts at 16.4 or 9.4 T (Bruker AVANCE). Xenografts derived from s.c. implantation of in vitro cultured SKOV3.ip cells (1x 10^6) in the dorsum of SCID mice were treated daily with the PC-PLC inhibitor tricyclodecan-9-yl-potassium xanthate (D609, 1 mg/mouse x 9 days) or saline (SAL), starting from day 7 post injection (dpi)) and their growth was monitored twice a week by caliper.

In vivo MRI/MRS measurements were performed on a Varian Inova system, operating at 4.7 T. MRI/MRS and a combination of volume a

Discussion and Conclusions - We here report the first evidence of a binding of PtdCho-PLC to HER2 in ovarian cancer cells. Moreover, the PtdCho-PLC activation status could play a role in controlling HER2 overexpression in SKOV3.ip cells. The here reported decreases in the in vitro cell proliferation and in the in vivo tumour growth following PtdCho-PLC inhibition suggest that this enzyme plays an important role in HER2-driven EOC cell signalling and tumorigenicity.

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References:

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<thead>
<tr>
<th></th>
<th>tCho (mM)</th>
<th>T2 (ms)</th>
<th>ADC (mm^2/s)</th>
<th>Ki67(%)</th>
<th>HER2 (score)</th>
</tr>
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<tbody>
<tr>
<td>SALINE (n=6)</td>
<td>4.2 ± 1.4</td>
<td>66 ± 15</td>
<td>8 ± 0.9</td>
<td>80 ± 10</td>
<td>strong</td>
</tr>
<tr>
<td>D609 (n=3)</td>
<td>b. d.</td>
<td>111 ± 17</td>
<td>12.8 ± 1.0</td>
<td>60 ± 26</td>
<td>moderate</td>
</tr>
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b.d., below detection.

**p<0.05
***p<0.01

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