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.

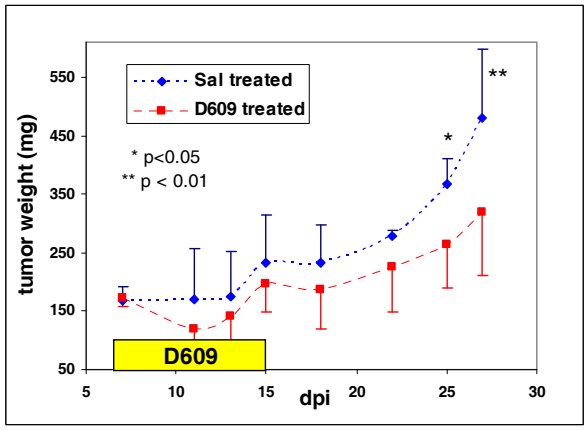
Methods - Cells: SKOV3ip cells were established from the in vivo passaged HER2-overexpressing SKOV3 cell line, as described in ref. 4. High resolution MRS analyses were performed on cell and tissue extracts at 16.4 or 9.4 T (Bruker AVANCE). Xenografts derived from s.c. implantation of in vitro cultured 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 and surface coil (RAPID Biomedical). MRI evaluation was performed by T1W (TR/TE=500/20ms) and T2W (TR/TE=3000/70ms) multislice spin echo images. ADC measurements were performed by acquiring DW images (TR/TE=2000/50 ms, b ranging from 123 to 1105 s/mm^2). Quantitative ^{1}H MRS analyses were performed according to a protocol described in [4] by using a PRESS sequence (TR/TE =4000/23 ms). LCModel was used for spectral fitting. Histological analysis of xenograft sections following hematoxylin/eosin, Ki67 and HER2 staining was performed on biopsies during and at the end of treatment.

Results -Separation of lipid rafts by sucrose gradient and immunoprecipitation experiments allowed detection of co-localization and physical association of PtdCho-PLC with HER2 in non-raft domains of in vitro-cultured SKOV3.ip cells. PtdCho-PLC inhibition in SKOV3.ip cells exposed to D609 (24h) was associated with a significant decrease in the PCho level down to 35-40% of control (untreated) cells, with a long-lasting block of cell proliferation (tested for up to 100h) - an effect similar to that exerted by serum deprivation – and with a strong reduction in the overall HER2 content measured in total cell lysates [Paris et al, manuscript in preparation].

In vivo experiments showed significant differences in the pattern of tumor growth of D609- vs. SAL-treated SKOV3.ip xenografts (ANOVA repeated mesasurements, $P<0.05$) (see Figure). Multiple posthoc comparisons evidenced a significant difference between the volumes of D609- (n=7) and SAL-treated (n=6) xenografts at 25 dpi ($P<0.05$) and 27 dpi ($P<0.01$). Histological analyses showed an average reduction of Ki67 proliferation index (27%, p=0.07) and a strong reduction in HER2 content expression (score index from 3.0 to 1.3, P=0.011 strong to moderate).

Preliminary results of in vivo MRI /MRS examinations showed in a subset of D609-treated animals (n=3) a marked decrease in the tCho level and increases in the mean T2 and ADC values, along with reduced Ki67 index and HER2 content (see Table). Quantitative MRS analyses on tumor extracts confirmed a significant (about 41%) reduction of PCho content in D609-treated tumors (n =3) compared with SAL-treated controls (P=0.006).



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

b.d., below detection.

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 :

1) Podo F et al. *NMR Biomed* 2011;24:648-72.
2) Glunde K et al. *Nature Rev Cancer* 2011; 11:835-48.
3) Iorio E et al. *Cancer Res* 2010; 70:2126-35.
4) Canese R et al. *NMR Biomed* 2012; 25 :632-42.