### 709 SOX2 expression is repressed by the BMP pathway and H.pylori in AGS gastric cell line

V. Camilo[1], R. Barros[1], A. Magalhães[1], L. David[1], R. Almeida[1]. [1]*IPATIMUP – Institute of Molecular Pathol, Carcinogenesis, Porto, Portugal*

**Background:** Infection with Helicobacter pylori (Hp) may trigger the development of intestinal metaplasia (IM), a preneoplastic lesion of the stomach characterized by a phenotypic switch from a gastric to an intestinal phenotype. Ectopic expression of CDX2 *per se* was sufficient to induce IM both in the stomach and other tissues. CDX2 is not expressed in the normal gastric mucosa therefore mechanisms of CDX2 repression may be lost in the progression to IM. One candidate for CDX2 repression is the transcription factor SOX2, since CDX2 and SOX2 expression in the gastrointestinal tract are mutually exclusive, with SOX2 being expressed in the esophagus and stomach and CDX2 in the intestine. Independent studies have shown that CDX2 is positively regulated by Hp and Bone Morphogenetic Proteins (BMPs) in the gastric context. Our aim was to evaluate if Hp and BMP/SMAD were negatively regulating SOX2 expression.
**Material and Methods:** (I) *BMP treatments:*AGS gastric carcinoma cells were treated with BMP-2 or BMP-4 or vehicle for 24 h. CDX2 and SOX2 expression were assessed by Real-Time PCR. For Western Blotting analysis, BMP-treated AGS cells were submitted to SDS-PAGE and incubated with polyclonal anti-SOX2 antibody. (II) *SMAD4 RNAi:* SMAD4 Knock-down cells were established using a retroviral short hairpin RNA expression system. CDX2 and SOX2 expression of SMAD4 RNAi AGS cells was assessed by Real-Time PCR. (III) *SMAD6/7 transfection*: AGS cells were transfected with SMAD6 or SMAD7 expression vectors using lipofectamine 2000. Cells were collected 48h after transfection for SOX2 and CDX2 expression assessement by Real-Time PCR. (IV) *Hp co-cultures:*AGS cells were co-cultured with Hp cagPAI negative and cagPAI positive strains and collected 8 hours post-culture. SOX2 expression was assessed by Real-Time PCR.
**Results:** In AGS cells, BMP treatment led to a decrease in the SOX2 levels, both at the RNA and protein levels. This tendency was reverted upon inhibition of the BMP canonical effector SMAD4. Transfection with BMP/SMAD inhibitors SMAD6 and SMAD7 increased SOX2 RNA levels. Finally, Hp infection decreased SOX2 RNA levels, independently of the Hp cagPAI status.
**Conclusion:** BMPs and Hp downregulate SOX2 expression by facilitating loss of gastric differentiation and gain of intestinal differentiation in gastric cells.

### 710 Sarcosine induces up-regulation of HER2/neu in androgen dependent prostate cancer cells

M. Dahl[1], P. Bouchelouche[1], G. Kramer-Marek[2], J. Capala[2], J. Nordling[3], K. Bouchelouche[4]. [1]*University of Copenhagen Koege Hospital, Cancer & Molecular Imaging Unit Research Division of Clinical Biochemistry, Koege, Denmark*, [2]*National Cancer Institute National Institutes of Health, Molecular Targeting Section Radiation Oncology Branch, Bethesda Maryland, USA,* [3]*Herlev Hospital Copenhagen University, Department of Urology, Herlev, Denmark,* [4]*Rigshospitalet Copenhagen University, PET & Cyclotron Unit, Copenhagen, Denmark*

**Background:** The oncoprotein HER2/neu is associated with invasiveness and proliferation of cancer cells. Additionally, there is increasing evidence that HER2/neu is also involved in prostate cancer progression. Sarcosine was recently reported as a novel biomarker for aggressive cancer and exogenous sarcosine induces an invasive phenotype in benign prostate cells [1]. Thus far, the pathways downstream HER2/neu and sarcosine are both elusive. The purpose of this study was to assess a possible relation between sarcosine and the expression of HER2/neu in prostate cancer cells. Insight into the progression of prostate cancer might pave the way for development of novel therapies.
**Materials and Methods:** Prostate cancer cells (LNCaP, PC-3 and DU145) were obtained from ATTC. Cells were exposed to 25–100 μM sarcosine for 24, 48 or 72 h. Relative amounts of HER2/neu and AR transcripts were determined using real-time quantitative reverse transcription PCR. Expression of HER2/neu and the androgen receptor (AR) was confirmed by Western blot. HER2/neu molecules displayed on the cell surface were probed using HER2/neu specific Affiprobes [2] and imaged by confocal laser scanning microscope.
**Results:** Exposure of LNCaP cells to 50 μM sarcosine for 24 h resulted in a 58% increase of the HER2/neu mRNA level ($P < 0.001$), indicating that addition of sarcosine influenced HER2/neu expression on the level of transcription. Up-regulation of HER/neu protein was confirmed by Western blot. However, no change in HER2/neu expression was found for the other cell lines studied. Control experiments with the sarcosine analogue alanine showed no significant effect on the HER2/neu mRNA level. The phosphorylated form of the HER2/neu protein, HER2/neu-P was also detected but sarcosine treatment did not confer any increase in activation of HER2. Imaging by confocal microscopy showed an increase of HER2/neu on the surface of LNCaP cells after 48 and 72 h of sarcosine treatment, with a more pronounced effect at the later time point.
**Conclusions:** This is the first report that exogenous sarcosine significantly increases HER2/neu expression in prostate cancer cells. Sarcosine seems to be involved in the regulation of HER2/neu on the level of transcription. Thus, sarcosine may induce progression and aggressivity of prostate cancer by increased HER2/neu expression.
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### 711 New phenol-substituted thiazolidinediones: an improved antiproliferative PPARgamma-independent effect on breast cancer cell lines

C. Colin[1], X. Yao[1], S. Kuntz[1], M. Boisbrun[2], Y. Chapleur[2], S. Flament[1], I. Grillier-Vuissoz[1]. [1]*Université Henri Poincaré, EA SIGRETO, Vandoeuvre-lès Nancy, France,* [2]*Université Henri Poincaré, UMR CNRS 7565, Vandoeuvre-lès Nancy, France*

**Background:** PPARs are ligand-activated transcription factors that belong to the nuclear receptor superfamily. Thiazolidinediones (TZD) are synthetic PPARγ agonists, which inhibit proliferation and migration of breast cancer cell lines. However, together wither other groups, we have shown that several anticancer effects of TZD do not require PPARγ activation. Despite hopeful results in preclinical studies, TZD display a low therapeutic index for breast cancer treatment. More potent and more selective compounds need to be developed. Therefore we synthesized new TZD, without PPAR? agonist activity and used biotinylation to improve the delivery of the molecules to cancer cells.
**Material and Methods:** The new compounds derived from an inactive analogue of Troglitazone (TGZ) named Δ2-TGZ. Biotin was added to the OH function of the phenol group (bΔ2-TGZ). The effects were studied in the hormone-dependent breast cancer cell line MCF-7 and in the hormone-independent cell line MDA-MB-231. Proliferation was studied using the "cell titer glo" test. Proteins were analyzed by western blotting and immuno-localisation.
**Results:** Our study shows that bΔ2-TGZ inhibited proliferation of both MCF-7 and MDA-MB-231 cells more drastically than TGZ or Δ2-TGZ. These data are correlated to cyclin D1 level since inhibition of proliferation is associated to cyclin D1 proteasomal degradation. MDA-MB-231 cells are more sensitive than MCF-7 cells. These effects are related to ER stress which is not triggered to the same extent by the different compounds as shown by DDIT3/CHOP expression.
In competition experiments, the presence of free biotin in the culture medium did not decrease the antiproliferative action of bΔ2-TGZ. Besides, other compounds that had no biotin but that were substituted at the same position of the phenolic group of the chromane moiety of Δ2-TGZ decreased cell viability similarly to bΔ2-TGZ. The relationship with ER stress is currently studied. These results suggest an important role of the OH substitution on the phenol group rather than a direct involvement of biotin.
**Conclusions:** Taken together, we developed new TZD compounds displaying a strong anticancer activity on both hormone-dependent and hormone-independent breast cancer cell lines. Their mechanism of action is not fully elucidated, but clearly the PPARγ-independent action is partially mediated by ER stress. These compounds could be important tools for developing new strategies for breast cancer treatment.

### 712 ICI 182,780 induces p-cadherin overexpression in breast cancer cells through chromatin remodelling at the promoter level: role of C/EBPbeta in CDH3 gene activation

A. Albergaria[1], A.S. Ribeiro[1], S. Pinho[2], F. Milanezi[1], B. Sousa[1], C. Oliveira[1], J. Machado[1], R. Seruca[1], J. Paredes[1], F. Schmitt[1]. [1]*IPATIMUP – Institute of Immunology and Molecular Pathology of Porto University, Cancer Genetics, Porto, Portugal,* [2]*Imperial College London, Hammersmith Hospital, London, United Kingdom*

**Background:** *CDH3*/P-cadherin is a classical cadherin which overexpression has been associated with proliferative lesions of high histological grade, decreased cell polarity and poor patient breast cancer survival. *In vitro* studies showed that it can be up-regulated by ICI 182,780, suggesting that the lack of ERα signalling is responsible for the aberrant P-cadherin overexpression and for its role in inducing breast cancer cell invasion and migration. However, the mechanism by which ER-signalling inhibition leads to P-cadherin expression is still unknown. The aim of this study was to explore the molecular mechanism linking the ERα-signalling and P-cadherin-regulated expression in breast cancer cell lines.