were analysed with genome-wide Methyl-Seq bisulfite sequencing. Five differently hypomethylated genes were selected using bioinformatics tools. Bioinformatic analysis was realised comparing colorectal primary tumour vs lymph node metastasis using methylKit tool.

**Results and discussions** A total of 196 genes were detected as differentially methylated in their promoter region, 94 of which were hypomethylated on lymph node metastasis group. CS, RNF130, HERC6, ZNF717 and RNF216-IT1 genes presented differences over 50% in their methylation status, compared with CRC primary tumours group. According to their ontology, these genes are involved in regulation of tricarboxylic acid cycle, transcription, carbohydrate metabolic process and protein polyubiquitination.

**Conclusion** CS, RNF130, HERC6, ZNF717 and RNF216-IT1 genes were found hypomethylated in colorectal metastasis compared to primary tumour. These genes could be implicated in metastasis behaviour in colorectal cancer, but further studies are necessary to evaluate their functions.

**PO-184 ROLE OF UBQUITIN-CONJUGATING ENZYMES IN CHROMOSOME INSTABILITY AND BREAST CANCER METASTASIS**

1F Salvador*, 1M Cejalvo, 1M Guix, 2E Fernández, 2L Pané, 2A Prat, 2R Gomis. 1Institute for Research in Biomedicine IRB Barcelona, Oncology, Barcelona, Spain; 2August Pi Sunyer Biomedical Research Institute IDIBAPS, Translational Genomics and Targeted Therapeutics in Solid Tumors, Barcelona, Spain

**Introduction** Chromosome Instability (CIN) is a hallmark in cancer being aneuploidy found in most of the tumours. In addition, high levels of CIN in primary tumours predict poor outcome in several cancer types. During last years, it has been demonstrated the role of aneuploidy during primary tumour generation in some transgenic mouse models. However, the role and functional consequences of CIN and aneuploidy during the metastatic process has not been deeply explored.

**Material and methods**
- Previous screening from the group (Gawrzak et al. 2018) identified several candidates genes potentially relevant in breast cancer (BC) dormancy.
- WB and IF were performed for analysis of mitosis and study of chromosome segregation.
- Cells were labelled with GFP-Luciferease for tracking tumour cells in mice.
- CIN70 signature was obtained by analysing RNA expression data from paired primary and metastatic tissues from BC (Cejalvo et al. 2017).

**Results and discussions** Taking advantage from a previous loss of function screening approach in dormant breast cancer cells we have identified an Ubiquitin-conjugating enzyme (UBE) as a candidate gene to control metastasis in BC. UBE has a pivotal role during cell division by controlling the stability of key mitotic players. UBE abrogation prolongs the spindle assembly checkpoint in several breast cancer cell lines, thus delaying mitosis exit. Further analysis shows that UBE depletion impaired the normal segregation of chromosomes during cell division increasing aneuploidy rates. Interestingly, in vivo studies with different breast cancer cell lines show an increase in the metastatic abilities of UBE downregulated cells. Additionally, we compared a signature of chromosomal instability (CIN70) between paired primary and metastatic tissues form BC. CIN70 score is clearly increased in metastasis, emphasising the importance of aneuploidy for the acquisition of the traits required for cancer metastasis in BC.

**Conclusion** Overall, our results suggest that CIN generated by disturbed levels of UBE increases cell plasticity to facilitate metastatic growth in BC.

**PO-185 TOWARDS DYNAMIC TARGETING OF TGF-β IN METASTATIC MELANOMA USING INTRAVITAL MICROSCOPY**

D Marvin*, S Van Gelderen, C Prunier, K Ammerlaan, P Ten Dijke, LMA Ritsma. Leiden University Medical Centre- Cancer Genomics Centre Netherlands- Oncode Institute, Cell and Chemical Biology, Leiden, The Netherlands

**Introduction** Melanoma patients diagnosed with liver metastasis have a poor prognosis. TGF-β inhibitors give mixed results in clinical trials with metastatic melanoma patients. To more specifically target TGF-β in liver metastasis melanoma patients, it is necessary to unravel the spatio-temporal function of the TGF-β pathway in hepatic colonisation of melanoma cells. TGF-β is a multifunctional cytokine that signals via TGF-β receptors and downstream SMAD effector proteins. SMAD proteins regulate transcription by binding, among others, to CAGA elements in the DNA. It can exert both pro- and anti-tumorigenic functions, depending on cellular context. It acts on tumour cells, as well as the tumour micro-environment and the immune system. In metastatic melanoma cells, TGF-β can stimulate invasion and metastasis. To unravel its exact role during the different processes of metastasis, we will investigate the spatio-temporal patterns of TGF-β signalling during metastatic colonisation using intravitral microscopy (IVM).

**Material and methods** Spatio-temporal patterns of TGF-β signalling will be studied in vitro and in vivo. For our in vitro studies, we are using an experimentally induced liver metastasis model, injecting highly aggressive B16F10 melanoma cells in immune competent C57BL/6 mice. By injecting these tumour cells in the mesenteric vein, cells will be transported directly to the liver, the first capillary network the cells will encounter. An abdominal imaging window will be placed after cell injection to visualise the different steps of metastasis in real-time using IVM. We developed a rapid CAGA12-GFP-based transcriptional reporter, which expresses a fluorophore upon TGF-β receptor activation and SMAD binding. Upon the expression of this reporter in B16F10 cells, activation of the TGF-β pathway can be monitored over time. By genetic manipulation of B16F10 cells to express dominant negative or constitutively active TGF-β receptors, the role of TGF-β can be assessed for the different steps of metastasis.

**Results and discussions** We confirmed earlier reports showing that B16F10 cells show a transcriptional CAGA response upon TGF-β stimulation. Using our liver metastasis model, the injected B16F10 cells are able to perform the steps of metastasis and form liver metastasis within a short time frame. The use of the rapid TGF-β reporter during intravitral imaging will show the involvement of the TGF-β pathway in the different phases of metastasis.
Conclusion B16F10 cells have a functional TGF-β pathway and are able to colonise the liver.

**PO-186** IN VITRO AND CLINICAL STUDIES OF THE ROLE OF MHC CLASS II INVARIANT CHAIN (CD74) IN BREAST CANCER

J. Boertmann-Noor*, A. Bartels, S. Fris, N. Brunner, J. Moreira, University of Copenhagen, Department of Drug Design and Pharmacology, Copenhagen, Denmark

10.1136/esmoopen-2018-EACR25.225

**Introduction** The major histocompatibility complex (MHC) class II invariant chain (CD74) is a protein which functions as a chaperone for MHC class II in antigen-presenting cells. Furthermore, it acts as a receptor for the cytosine macrophage migration inhibitory factor (MIF), mediating downstream signalling. Many clinical studies have determined that CD74 is overexpressed at the protein level in a subset of human breast tumours. However, there are discrepant findings regarding its correlation to clinical parameters. In some reports, CD74 has been correlated to triple-negative (TN) status and increased presence of metastases, but in separate studies it correlated with better overall survival. In vitro studies of CD74’s molecular functions in cancer cells have generally supported that it has a cancer-promoting effect through stimulating proliferation, survival and invasion.

**Material and methods** Human breast cancer cell lines MDA-MB-231 and MDA-MB-446 were transfected with siRNA targeting CD74 or a negative control. Cells were then subjected to in vitro analysis of proliferation or invasion through matrigel. Lysates of transfected cells were analysed by immunoblot for protein levels of cell survival and autophagy markers.

A tumour microarray (TMA) containing 651 human breast tumour cores was stained by immunohistochemistry (IHC) for CD74 protein expression. Staining intensity was manually scored for each sample in a blinded fashion. Statistical analysis was performed in R.

**Results and discussions** siRNA knockdown of CD74 in MDA-MB-231 and MDA-MB-446 resulted in reduced proliferation and invasion. Furthermore, survival signalling through Akt was decreased. These findings replicate previously published in vitro studies of CD74’s functions in cancer cells. Furthermore, a novel finding was that CD74 knockdown resulted in reduced levels of markers of autophagy.

The correlations between CD74 IHC staining of TMA samples and TN status, lymph node status and overall survival were examined. In contrast to previous studies we observed no correlation between expression of CD74 and TN status or cancer spread to the lymph nodes. Instead, survival analysis revealed increased overall survival for cancers with moderate or high CD74 intensity.

**Conclusion** Our results from cell lines support that CD74, in contrast to previous studies we observed no correlation to clinical parameters. In some reports, CD74 has been correlated to triple-negative (TN) status and increased presence of metastases, but in separate studies it correlated with better overall survival. In vitro studies of CD74’s molecular functions in cancer cells have generally supported that it has a cancer-promoting effect through stimulating proliferation, survival and invasion.