Recent studies point out that aberrant miR-34a and miR-137 expression leads to an increase in cell proliferation, as well as an abnormal response to chemotherapy in various types of cancer. Thus, we aimed to elucidate the role of this two microRNAs in ALL, specifically to study their association to tumor development and disease progression. To achieve this goal, we first analyzed the expression of these two microRNAs in B-ALL and T-ALL cell lines, using normal lymphoid cells as controls. We found lower expression of miR-34a and miR-137 by RT-qPCR in cancer cell lines compared to control cells. Furthermore, we studied the main proteins regulated by these non-coding RNAs: SIRT1 and LSD1. We demonstrated by Western Blot that SIRT1 and LSD1 levels were significantly higher in ALL cell lines. Finally, we overexpressed miR34a in the ALL cell lines by transfection with an expression vector in order to confirm that the lower expression of this microRNA is associated with the tumoral phenotype. The restored levels of miR-34a resulted in a downregulation of SIRT1 protein levels and cell proliferation. Thus, these novel results provide new insight into ALL cell biology. Furthermore, these results encourage us to continue studying the role of miR-34a and miR-137 as an early diagnostic molecule or a possible effective target for disease treatment.

**241.** (92) **ADIPOCYTES IN THE BREAST CANCER MICROENVIRONMENT: DIFFERENT TUMOR CELLS, DIFFERENT WAYS TO RESPOND**

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Adipocytes are considered to be critical in the tumoral microenvironment of breast cancer. However, most studies have focused on linking obesity and cancer, ignoring changes on normal adipocytes. Therefore, we retrieved microarray data from a GEO dataset (GSE95827) to analyse transcriptional changes in 3T3-L1 adipocytes after 3 days of co-culture with MCF7 (Ad+MCF) or MDA-MB-231 breast cancer cell (Ad+MDA).

By GEO2R tool, we determined 245 up-regulated genes in Ad+MCF7 and 215 in Ad+MDA compared to adipocytes alone ($p<0.01$), but only 68 of them overlapped between conditions. Gene Ontology Analysis was performed and showed that the biological process enrichment is completely different between MDA-MB-231 and MCF7-co-cultured adipocytes. Even more, co-culture with MDA-MB-231 cells enriched adipocytes with genes involved in *Cellular response to interleukin-6* (GO:0071354) and *Positive regulation of NIK/NF-κB signaling* (GO:1901224), both related to inflammation. Transcription factors (TF) analysis by ISMARA platform predicted that NF-κB family members had the highest activity in Ad+MDA. To verify this result, we performed immunofluorescence assays detecting the presence of phosphorylated NF-κB subunit p65. MDA-MB-231 cells significantly led to a higher fluorescence intensity in adipocyte nuclei than MCF7 did when compared to basal condition. Moreover, mRNA levels of NF-κB targets as *cxcl1, cxcl5, il6* obtained from the analysed dataset were found up-regulated in Ad+MDA respect to adipocytes alone ($p<0.05$). Interestingly, expression levels of Il6 family members (Il6 and Il11) were up-regulated in MDA-MB-231 cell line compared to MCF7 cells in three different public datasets, which could explain NF-κB activation only in Ad+MDA.

These results suggest that breast cancer cells stimulate adjacent adipocytes in different ways leading or not to inflammation in adipocytes.

**242.** (96) **TGF- B1 IMPLICATIONS ON LUMINAL-MYOEPITHELIAL DIALOGUE IN BREAST CANCER**

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The mammary gland duct is composed by an internal cell line formed by luminal cells (LEP) surrounded by an external one of myoepithelial cells (MEP). It is still unknown how these cells contribute to the progression of ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC). Some works suggest that TGF-β1 pathway may be involved in epithelial-mesenchymal transition and confer stem cell properties to DCIS cells. This implies that this pathway might be a potential pathological mechanism which drives the progression of DCIS into IDC.

The cellular model LM38 consists of three cell lines: LM38-LP (MEP and LEP), LM38-HP (LEP) and LM38-D2 (MEP). Previously, we described that only the bi-cellular LM38-LP cell line was able to develop in situ tumors after intraductal injections, suggesting that cell interaction could confer an advantage for tumor formation and progression. Moreover, we showed that treatment with conditioned medium of LM38-D2 induced viability on LM38-LP cells.

The analysis of TGF-β1 expression in the LM38 model showed higher levels of TGF-β1 mRNA in LM38-D2 compared to LM38-LP (qPCR, $p<0.01$). LM38 cells were treated with a recombinant TGF-β1 (1ng/$\mu$l) and an inhibitor of the TGF-β1 receptor SB431542 (10 and 20$\mu$M). We could observe that TGF-β1 treatment increased 40 per cent the viability of LM38-LP compared to the control, which is reduced in presence of SB431542 (crystal violet assay, $p<0.05$, $p<0.001$).

When evaluating TGF-β1 expression in LM38 fat pad tumors we observed that tumors generated by LM38-D2 showed higher expression levels on TGF-β1 than LM38-LP (IHQ, p=0,01). Expression of TGF-β1 presented an heterogeneous pattern in LM38-LP tumors which requires further characterization.

In conclusion, TGF-β1 could be one of the factors implicated in the LEP-MEP dialogue. These results suggest that TGF-β1 could play an important role in cellular cooperation in early stages of breast cancer.

**243.** (98) **CHARACTERIZATION OF AN IMATINIB-RESISTANT CML K562 CELL LINE: KI562. EFFECT OF 4-METHYLUMBELLIFERONE ON ITS METABOLIC ACTIVITY AND CD44 EXPRESSION.**

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CML is a myeloproliferative neoplasia whose first-line therapy are BCR-ABL inhibitors such as Imatinib (IM). CD44 levels correlates with bad response to therapy. Previously, we demonstrated that hyaluronic acid (HA) abrogates IM-induced senescence, while the inhibition of its synthesis with 4-methylumbelliferone (4MU) has a synergistic effect with IM on CML cells growth. The aim of this work was to obtain an IM resistant K562 derivate cell line and to study the resistance mechanisms involved, as well as, the effect of 4MU treatment. The Ki562 cells were obtained after culturing K562 cells with increasing doses of IM from 0.1$\mu$M up to 1$\mu$M. Control cells derivate of K562, Ko562, were kept in culture presenting the same aging but, without selection pressure of IM. None of these cells showed efflux pump activity (determined by flow cytometry, FC). Both cell lines had a similar frequency of the F359I mutation (evaluated by DNA sequencing). However, Ki562 cells showed higher levels of *BCR-ABL* than Ko562 cells (evaluated by qRT-PCR and WB, $p<0,01$). Both of them, expressed similar levels of surface CD44 (evaluated by FC), which was downregulated by 4MU as well as by IM only in Ko562 cells ($p<0.05$). 4MU decreased the metabolic activity on both cell lines (determined by XTT, $p<0.01$) without modifying the percentage of PI+ cells respect to untreated control (evaluated by FC). Moreover, the co-treatment with 4MU+IM inhibited metabolic activity more than each drug alone in both cell lines ($p<0.01$), without modifying the percentage of PI+ cells. We conclude that Ki562