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Migration and invasion are the main tumor attributes that allow to distinguish between primary and metastatic tumors. Osteosarcoma (OS) is the most prevalent bone tumor, mostly affecting pediatric patients. One of its main clinical challenges is rapid metastatic spread, raising the importance of highlighting the cancer hallmarks: migration and invasion. In order to assess the tumor niche contribution to migration, we carried out assays with mesenchymal stem cells (MSC) and microvascular endothelial cells responding to primary and metastases OS cell lines-conditioned media (CM), demonstrating that both stromal cells migrated further towards the primary OS secretome. Afterwards we analyzed OS cell lines SAOS2 and LM7 migration to MSC-CM, resulting in LM7 migrating significantly more to MSC secretome. With these results in mind, we iv. inoculated OS cells into athymic mice, removed the lungs, obtained lungs-CM and challenged MSC to these CM. Surprisingly, MSC migrated significantly more to CMs from the lungs with LM7 cells. Since this difference could be attributed in part to the complexity added by the in vivo lung microenvironment scenario, we analyzed the dataset GSE14359. Of interest, S100A14 and PECAM1 showed significantly higher expression in metastatic samples compared to the primary counterpart, while CXCR4 and IL-1α showed a similar trend. We also demonstrated a homing of MSC in OS lung metastases. Tissue remodeling is fundamental to allow migration from the primary tumor. We analyzed MMP-2/9 expression and activity, demonstrating presence of pro and active MMP-2 and absence of MMP-9. Of relevance, a higher MMP-2 expression is associated with a worse overall survival time in OS patients. Among other members involved in this biological process, cathepsin D was upregulated in metastatic OS cells. Lung disease remains a major OS death cause. Identification of differentially expressed genes would uncover promising markers and therapeutic approaches for OS spread.

605. (110) **NOVEL METHYLATION-BASED BIOMARKERS FOR COLORECTAL CANCER DETECTION**

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Epigenetic marks refer to chemical DNA modifications that do not affect the DNA sequence but can modify gene expression. They are described early in the tumorigenic process and can be used as biomarkers, for example, for early cancer detection. We developed a bioinformatic algorithm that identifies and hierarchies sensitive and specific epigenetic biomarkers. Currently, we are developing a PCR-based technology capable of detecting biomarkers with high sensitivity, either individually or combined in the same reaction. Objective: Identify and detect methylation-based biomarkers in DNA extracted from tumors derived from colorectal cancer (CRC) patients. Methods: We developed a bioinformatic platform capable of implementing an algorithm that identifies methylation-based biomarkers from public databases (TCGA). We constituted an average risk CRC patients prospective cohort, who attended for video colonoscopy and/or CRC resection surgery. We isolated DNA from the tumor and paired colonic normal tissue distant from the lesion. We used a PCR-based technology to detect a well know epigenetic biomarker (SEPT9) for CRC screening and our proprietary biomarkers. Results: initially, we validated the bioinformatic platform since it accurately identified already described biomarkers and we also identified our own panel of new biomarkers. Applying our own technology, we detected SEPT9 methylation in DNA tumor tissue patients from 20 CCR patients. We applied a ROC analysis obtaining an Area Under the Curve (AUC) of 0.98, which means a high sensitive and specific detection. As controls, we used paired colonic normal tissue. Finally, we chose the best ranked biomarkers identified by the bioinformatic platform that were detected in the same samples, either individually or combined as a biomarker panel in the same PCR reaction (AUC=0.995). Conclusion: We identified and detected a panel of new methylation-based biomarkers that might be implemented in colorectal cancer screening.

606. (191) **DIRECT ACTION OF INTESTINAL MICROORGANISMS IN COLORECTAL CANCER CELL LINE**

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Numerous evidence demonstrate the importance of the microbiome mainly due to its immunological action, in the development of multiple diseases, such as colorectal cancer (CRC). In agreement with previous works, bacteria such as *Escherichia coli* (*E.coli*) and *Bacteroides fragilis*, among others, have high prevalence in CRC patients compared to the normal population. In this work we investigate the possible pathways and signaling that may contribute to CRC development that could be affected by direct action of bacteria over colorectal cancer cells, in the absence of additional signals from the immune system. Therefore, we first determined the total amount of genes that were up or down regulated (log FC >1 or <-1), as determined by RNAseq, in experiments where the CRC cell line DLD-1 was infected with the bacteria *E.coli* for 2 h or *Enterotoxigenic Bacteroides fragilis* (ETBF) for 2 to 24 h at MOI: 500 (database GSE130152). Then, we performed an over-representation analysis using the ConsensusPathDB bioinformatic tool. We found that ETBF upregulates 525 genes involved in processes like cell migration, inflammatory response, and the FOXO-mediated transcription of cell cycle genes (at least $p < 0.05$). While *E.coli* upregulates 2249 genes, most of them involved in TNF, NF-kB, MAPK, and inflammatory signaling pathways (at least $p < 0.05$). Interestingly, 231 upregulated and 414 downregulated genes are shared with ETBF as determined by Venn diagrams. Then to confirm the previous suggested incidence of these bacteria in CRC, we analyzed the presence of both, using LDA score (linear discriminant analysis) in colorectal neoplasms from GMrepo database of human gut metagenomes and found that they have LDA>4, thus, strongly involved in CRC.
We conclude, there are signals and pathways independent of the immune system that could be induced directly by bacteria in the epithelial colorectal cells, contributing to CRC.

607. (301) **TOWARDS A BRAND NEW WAY TU UNDERSTAND KIDNEY CANCER: AN UNSUPERVISED MACHINE LEARNING APPROACH**

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