found that Pikkc2a loss causes an aberrant microtubule (MT) spindle organization that, in turn, promotes genomic instability. In line with this, Pi3kc2a is specifically enriched at the metaphase spindle, interacting with transforming acidic coiled-coil protein 3 (TACC3)/colonic, hepatic tumor overexpressed gene (ch-TOG)/clathrin complex to stabilize K-fibers during mitosis. Despite the aberrant MT organization, we demonstrate that tumors bypass the requirement of Pi3kc2a through a common mechanism of progression. Multiple genes involved in the spindle-associated checkpoint (SAC), such as Bub1, Bub3 and APC/C genes, resulted either amplified or lost in fast growing compared to slow growing tumors. In addition, tumors with low Pi3kc2a and aberrant spindle organization showed increased sensitivity to anti-MT agents (Paclitaxel) both in vitro and in vivo. Expression profiles of breast cancer patients showed that reduced levels of PIK3C2A correlated with aggressive tumors, indicating that reduction in PIK3C2A expression provides a growth advantage in mice as well as in patients.

Conclusions. We demonstrated that loss of PI3KC2A plays a crucial role in promoting genomic instability and sensitivity to anti-mitotic agents. These findings will eventually validate PI3KC2A as a new diagnostic/prognostic tool that can be exploited to tailor more effective therapies for breast cancers.

No conflict of interest.

319 Concomitant intracellular retention of SPARC and CATHEPSIN B by SCD5-induced oleic acid production reduces melanoma malignancy

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Introduction. The increased demand of fatty acids (FA) to assemble the plasma membranes of continuously dividing cancer cells might unbalance their ratio and critically affect tumor outgrowth. Saturated FAs, as palmitic and stearic, are desaturated by Δ9 stearoyl-coA-desaturase enzymes (SCD1 and SCD5) to produce the monounsaturated palmitoleic and oleic FAs. The effect of desaturase activity in cancer cells is partially known for SCD1, while virtually unknown for SCD5. We have investigated the expression and function of SCD5 in melanoma.

Materials and Methods. Expression studies were performed by western blot, qReal time PCR, immunohistochemistry and immunofluorescence according to standard procedures. Quantification of FAs was evaluated by gas chromatography/mass spectrometry analysis. For in vivo studies, xenografted nude mice were utilized to evaluate SCD5 functional role on tumor growth and metastatic potential. The effects of different pH culture conditions were evaluate.

Results and Discussion. The close correlation between the expression pattern of SCD5 and the amount of oleic acid (OA), both downmodulated during melanoma progression, suggests their role against the aggressiveness of advanced melanoma. Accordingly, SCD5 enforced expression in A375M metastatic melanoma, besides inducing a significant increase of OA, blocks SPARC release leading to its significant intracellular accumulation paralleled by cathepsin B and collagen IV retention. The same results on the malignant parameters of melanoma were obtained by the exogenous supplementation of OA. More important, in vivo models of induced human melanoma metastases or murine spontaneous metastases confirmed a significant SCD5-dependent impairment through a process that modifies the ECM. Finally, results indicated that the SCD5- or OA-dependent reduction of the intracellular pH is associated with a more physiological condition (pHext > pHint) according to the notion that an acidic microenvironment enhances SPARC activity and ECM remodeling toward dissemination.

Conclusion. Our data support a protective role of SCD5 and its enzymatic product oleic acid against malignancy, a finding offering explanation for the beneficial Mediterranean diet. Furthermore, SCD5 appears to functionally connect tumor cells and surrounding stroma toward modification of tumor microenvironment with consequences on tumor spread and resistance to treatments.

No conflict of interest.

320 Tumor-associated stromal cells increase malignancy of human colorectal cancer inducing epithelial-to-mesenchymal transition

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Introduction. During tumor formation, normal tissue microenvironment is transformed in an 'altered' niche, composed of non-malignant supporting cells, which influence the homeostasis of cancer cells via paracrine regulators. Tumor-associated stromal cells (TASC) are the prominent stromal elements in most types of human carcinomas including colorectal cancer (CRC). The differentiation of TASC from other cell types, such as resident stromal cells or bone marrow-derived mesenchymal stem cells (BM-MSC) is mainly mediated by factors produced during the crosstalk with tumor. TASC produce various extracellular matrix proteins, chemokines, and other promoting factors which affect vascularization, tumor cell proliferation and invasiveness, and they also play a critical role in determining response to therapy. TASC-derived factors may contribute to the development of a protective milieu by influencing cell-cell/cell-matrix interactions, cell survival, and suppression of anti-tumor immune responses. Moreover, physical contact between TASC and malignant cells supports cell survival via activation of anti-apoptotic pathways or inducing epithelial-to-mesenchymal transition (EMT). In this scenario our purpose is to address phenotypic and functional characterization of TASC in vitro and analyzed TASC-mediated effects on CRC development and progression in vivo.

Material and Method. TASC were characterized for phenotype and differentiation capacity. Human CRC cells were cultured in the presence of TASC in 2D and in 3D perfused bioreactor, sorted by flow cytometry and evaluated for the expression of EMT-related genes by Real Time PCR and for in vitro invasiveness by chemoinvasion assay. Furthermore, their tumorigenicity was assessed upon injection in NOD/SCID mice and developing tumors were analyzed.

Results. Our results indicate that TASC freshly isolated from CRC samples comprise a multipotent subpopulation that is able to differentiate into adipogenic and osteogenic lineage. After coculture with CRC cells they express membrane-bound TGF-beta, through which they are capable to trigger EMT. Moreover CRC cells cocultured with TASC acquire an elongated shape and a more invasive phenotype. Upon subcutaneous injection in NOD/SCID mice, tumor cells cocultured with TASC show a significantly faster growth kinetic and develop significantly larger tumor masses with an higher vessel density as compared to tumor cells alone. Interestingly tumors developed from tumor cells cocultured with TASC display the presence of LGR5 positive cells.

Conclusion. Thus our data show that the stromal component of CRC comprises a multipotent subpopulation and increases the tumor cells malignancy triggering EMT induction through membrane-bound TGF-beta.

No conflict of interest.

321 Nrf2, but not ß-catenin, mutation represents an early event in rat hepatocarcinogenesis

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Introduction. Hepatocellular carcinoma (HCC) develops through a multistage process but the nature of the molecular changes associated to the different steps, the very early ones in particular, is largely unknown. Recently, dysregulation of the NRF2/KEAP1 pathway and mutations of these genes have been observed in experimental and human tumors, suggesting their possible role in cancer development.

Material and method. To assess whether Nrf2/Keap1 mutations are early or late events in HCC development, we investigated their frequency in the Resistant-Hepatocyte (R-H) model, a chemically-induced rat model of hepatocarcinogenesis, analyzing preneoplastic and neoplastic liver lesions by Sanger sequencing.

Results and discussion. We found that Nrf2/Keap1 mutations were present in 71% of early preneoplastic lesions and in 78.6% and 59.3% of early (eHCC) and advanced HCC (aHCC), respectively. Nrf2 mutations were more frequent, missense and involved the Nrf2-Keap1 binding region. Mutations of Keap1 occurred at a much lower frequency in both preneoplastic lesions and HCCs and were mutually exclusive with those of Nrf2. Unlike Nrf2, mutations of Ctnnb1, which are frequent in human HCC, were a later event, as they appeared only in fully advanced HCCs (18.5%). Functional in vitro experiments show that Nrf2/Keap1 mutations lead to pathway activation, as demonstrated by the strong upregulation of NRF2 target genes (Nqo1, Gclc, Gsta4) in mutated preneoplastic lesions compared to control liver or to non-mutated preneoplastic nodules, suggesting that these are, actually, activating mutations. We found a strong upregulation of Nqo1 in eHCCs and aHCCs compared to normal liver, as well. Interestingly, unlike preneoplastic lesions, no significant difference in Nqo1 expression was found in mutated vs. non mutated HCCs, suggesting that, at late stages, the Nrf2-Keap1 pathway no longer depends on the presence of activating mutations only, but it can be activated by other mechanisms. In vivo studies showed that Nrf2 silencing inhibited the ability of tumorigenic rat cells to grow in soft agar and to form tumors, if subcutaneously injected in syngeneic rats.

Conclusion. Our results demonstrate that in the R-H model of hepatocarcinogenesis, the onset of Nrf2 mutations is a very early event, likely essential for the clonal expansion of preneoplastic hepatocytes to HCC, while Ctnnb1 mutations occur only at very late stages. Moreover, functional experiments demonstrate that Nrf2 is an oncogene, critical for HCC progression and development.

No conflict of interest.

322 Inhibition of CXCR4 receptor by a novel peptide antagonist modulates microglia reactivity and angiogenesis in a human glioblastoma model

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Background. The chemokine receptor CXCR4 is widely expressed in cancer. Its activation by the chemokine CXCL12 has been shown to sustain metastasis and angiogenesis and regulate the crosstalk between tumor and microenvironment. In