growth and maturation, mammary gland development, and its susceptibility to transformation.
Material and Methods: Female Sprague-Dawley rats were fed a low-fat, high corn oil (HCO) or high extra-virgin olive oil (EVOO) diet from weaning and gavaged with 7,12-dimethylbenz[a]anthracene. Animals were euthanized at 24, 36, 51, 100 and 246 days. We evaluated several parameters of growth and sexual maturation, as well as the clinical manifestation of mammary carcinogenesis.
Results: The administration of the HCO diet, but not the high EVOO diet, increased the body weight and mass of the animals. The vaginal opening was advanced in both high-fat groups, especially in HCO. This HCO group also had increased body weight around puberty, more corpora lutea at post-puberty, and tended to have higher mRNA levels of kisspeptin in the hypothalamus, a marker of sexual maturity. Both high-fat diets induced subtle modifications in the morphology of the mammary gland, with no changes on β-casein or hormone receptors expression in the gland. The HCO diet had a clearly stimulating effect of the carcinogenesis, inducing the earliest appearance of tumors and the highest tumor incidence and yield, whereas the high EVOO diet seemed to have a weak enhancing effect, increasing tumor yield.
Conclusion: Our data suggest a strong influence of HCO diet in sexual maturation and mammary cancer risk, while rats fed the high EVOO diet were more similar to the controls. Moreover, the data highlight the transcendence that dietetic factors may have on health and the importance of establishing healthy dietetic habits from childhood.

728 The Role of FOXM1 and NBS1 in DNA Double Strand Breaks Repair and Epirubicin Resistance

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Background: Dysregulated forkhead box M1 (FOXM1) expression is associated with epirubicin resistance in breast cancer and this can occur through an enhancement of DNA damage repair. However, it still remains unclear how FOXM1 modulates DNA repair and the mechanism involved.
Materials and Methods: Using DNA damage and repair assays, we studied the role and regulation of FOXM1 and NBS1 in breast cancer drug resistance and sensitivity.
Results: Here, we demonstrated that the protein levels of FOXM1 and NBS1, which is required for activation of ATM in response to DNA double stranded break (DSBs) repair, to be higher in the epirubicin-resistant MCF-7 breast carcinoma (MCF-7-EPI^R) cells compared with the parental MCF-7 cells. Interestingly, the knockdown of FOXM1 by siRNA transfection significantly decreased NBS1 mRNA level in many cancer cell lines and human fibroblasts. Moreover, foxm1−/− mouse embryonic fibroblasts also displayed reduced protein expression of NBS1 compared with wild-type mouse embryonic fibroblasts. Using DR-GFP HeLa cells, we found that depletion of FOXM1 impairs the homologous recombination-mediated DNA double stranded break repair. Furthermore, we found that foxm1−/− mouse embryonic fibroblasts transfected with wild-type FOXM1 exhibited decreased DNA breaks after epirubicin treatment, as evidenced by immunofluorescence focus staining of γH2AX, compared with foxm1−/− mouse embryonic fibroblasts transfected with control plasmid.
Conclusions: Taken together, our results indicate that FOXM1 mediates DNA double stranded break repair through the regulation of NBS1 expression and ATM activation.

729 FOXM1 Regulates BRIP1 Expression in Breast Cancer Epirubicin Treatment and Resistance

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Background: Breast cancer is the most common malignancy in women, with 1 in 9 of all British and American women developing this disease in their lifetimes. Chemotherapy with anthracyclines, particularly epirubicin, plays a key role in the medical management of breast cancer. The Forkhead box M1 (FOXM1) is ubiquitously expressed in proliferating cells and its deregulation is associated with cancer progression and development of cancer drug resistance. The aim of this work is to unravel the role of FOXM1 in response to epirubicin-induced double strand breaks (DSB) in breast cancer sensitive and resistant cell lines.
Materials and Methods: Sensitive MCF-7 and MCF-7-Epirubicin resistant (MCF7-EPI^R) cell lines were treated with epirubicin. We compared FOXM1 levels in response to epirubicin by means of Western blot and real-time quantitative PCR analysis. For the analysis of epirubicin-induced DNA damage and the influence of the drug on its repair, comet assay and immunofluorescence microscopic detection of the phosphorylated form of histone variant H2AX (γH2AX) foci were used. To analyse the capacity of FOXM1 to transactivate BRIP1 promoter and to determine the FOXM1 ability to directly bind to BRIP1 promoter, luciferase and chromatin immunoprecipitation (ChIP) assays were conducted, respectively. To determine the repair pathway these genes are involved in, we performed Homologous Recombination (HR) repair assay.
Results: FOXM1 expression levels are maintained high in epirubicin resistant MCF-7-Epi^R cells and downregulated in sensitive MCF-7 cells following epirubicin treatment and showed a close correlation with expression of the DNA double strand break repair protein BRIP1. The MCF-7-Epi^R but not the parental MCF-7 cell line shows absence of DNA damage upon epirubicin by λH2AX foci and comet assay, furthermore, silencing of FOXM1 reverses epirubicin resistance in MCF-7-Epi^R cells and the stable FOXM1 MCF-7 cell line is able to overcome sensitivity to the same drug. Moreover, reconstituting FOXM1 in *foxm1*$^{-/-}$ mouse embryonic fibroblasts, reduces the number of foci when compared to non-transfected cells, further confirming that FOXM1 has an active role in mediating resistance to epirubicin, by enhancing repair pathways. Indeed, the knockdown of FOXM1 and BRIP1 by siRNA results in accumulation of DSBs, due to decreased repair by HR. A reporter gene assay shows that FOXM1 activates BRIP1 transcription through a forkhead-response element (FHRE) located within the proximal promoter region. The direct binding of FOXM1 to the BRIP1 promoter is confirmed in vivo by ChIP analysis.
Conclusions: Together, these data demonstrates that FOXM1 mediates epirubicin resistance in breast cancer in part, by transcriptionally activating DNA damage repair proteins such as BRIP1.

730 Analysis of EMSY in Italian Male Breast Cancer Patients

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Background: Male breast cancer (MBC) is a rare disease compared to female breast cancer (FBC). MBC shares many similarities with FBC, including genetic predisposition factors such as *BRCA1/2*, *CHEK2, PALB2, BRIP1* and *RAD51C* mutations. However, these alterations can explain only 10% of MBC cases, thus suggesting the contribution of additional susceptibility genes. *EMSY* has been recently identified as a gene involved in FBC pathogenesis because EMSY can interact with BRCA2 and in this way it is capable of silencing the activation potential of BRCA2. Moreover, breast tumors with amplified *EMSY* show a phenotypic profile that is similar to *BRCA2*-related tumors. So, because of the interaction between *BRCA2* and *EMSY*, the latter could play a relevant role in MBC and could explain those MBC cases which pathogenesis can't be related to *BRCA1*/*BRCA2* mutations.
To date, there are no information about the role of *EMSY* in the pathogenesis of MBC. Taking into account that *EMSY* has a prognostic value for FBC, studies on its role could have important implications in the elucidation of pathogenetic mechanisms of MBC and in the clinical management of MBC patients.
Material and Methods: This study was performed on a series of 100 MBC cases characterized for *BRCA1*/*BRCA2* germ-line mutations and for relevant clinicopathologic features. We have investigated the presence of germ-line mutations and amplification of *EMSY* by automatic sequencing and qRT-PCR respectively. Statistical analysis was performed using the Fisher exact test.
Results: We have found *EMSY* alterations in 5% of our series. Three of the 37 variants identified (M83K, M1197I and IVS5−1G>A) were shown to be probably damaging by using two prediction softwares. We have found a general amplification percentage of 44% and we have distinguished three different amplification subgroups. A statistically significant association emerged between *EMSY* amplification and MIB1 ($p = 0.03$) expression.
Conclusions: Our data indicate that alterations of *EMSY* are involved in MBC pathogenesis at a comparable level as in FBC. New coding variants of this gene seems to be involved in MBC pathogenesis and *EMSY* amplification allows the identification of distinct subgroups of MBC cases. Moreover, although larger studies are needed, our results suggest that *EMSY* could be involved not only in MBC pathogenesis but also in tumor progression.
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731 Gene Copy Number Alterations in Male Breast Tumors

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Background: To date knowledge about specific biological and molecular characteristics of male breast cancer (MBC) is almost not existent, thus it's difficult to identified different subclasses that have both biological and clinical relevance, as observed in female breast cancer (FBC).
Gene copy number (GCN) alteration is a common mechanism of oncogenic activation in breast cancer (BC). We aimed to analyze GCN variation of genes involved in cell proliferation, hormone metabolism and cell cycle control, that