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

are know to play a relevant role in FBC as prognostic factors and as possible targets for therapy and that may play also a role in MBC. In particular we analyzed GCN alterations of *EGFR, PIK3CA, ESR1, CCND1* and *SULT1A1,* in order to identified new biomarkers in MBC that may allow to better understand the pathogenesis of MBC and can lead to the identification of MBCs subgroups with specific clinical-pathologic characteristics.

Material and Methods: GCN alterations of *EGFR*, *PIK3CA*, *ESR1, CCND1* and *SULT1A1* were evaluated on a series of 100 MBC tumors characterized for *BRCA1/2* mutations, the major genetic risk factor, and for relevant clinical-pathologic features. The analysis was performed by TaqMan assay using Real-Time PCR.

Results: Overall, *PIK3CA* showed an amplification frequency of 8.5%, *EGFR* of 9%, *CCND1* of 15% and SULT1A1 of 4.2%, whereas *SULT1A1* and *ESR1* were deleted with a frequency of 14%. Significant statistically association emerged between *PIK3CA* amplification and HER2 expression ($p = 0.023$), *EGFR* amplification and ER− status ($p = 0.01$), HER2 and MIB1 expression ($p = 0.026$ and 0.013) and T4 ($p = 0.027$). A significant statically association emerged also between *ESR1* deletion and ER− status ($p = 0.02$), *CCND1* amplification and HER2 ($p = 0.017$) and MIB1 expression ($p = 0.03$) and between *SULT1A1* deletion and ER− status ($p = 0.015$) and G3 ($p = 0.03$). These data suggest that *EGFR, CCND1* and *SULT1A1* alterations may be linked to an aggressive phenotype in MBC.

Conclusions: Our results indicate that the presence of *EGFR*, *PIK3CA, ESR1, CCND1* and *SULT1A1* GCN alterations can lead to the identification of MBCs subgroups with specific clinical-pathologic characteristics that can be useful in clinical management of MBC patients.

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732 The p38 MAPK2-MK2 Signalling Axis is Central in the Regulation of E2F1 and FOXM1 by Epirubicin

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Introduction: Elevated levels of FOXM1 are often associated with the initiation and progression of many types of cancers. In particular, FOXM1 has been reported to have a critical role in the determination of chemotherapeutic drug sensitivity. It was shown that the responsiveness of anthracycline works via the repression of FOXM1 expression which is dependent on the activity of E2F1.

Materials and Methods: The depletion of E2F1 expression was done using siRNA to determine the effects of FOXM1 activity upon epirubicin treatment. Futher inhibition studies were done on p38 using pharmalogical inhibitors, siRNAs and knockout MEFs. Phosphorylation assays were done to demonstrate the link between MK2 and E2F1.

Results and Discussion: We have shown that E2F1 is critical in the regulation of FOXM1 expression since its depletion by siRNA significantly affected FOXM1 induction and cell viability in response to epirubicin. Interestingly, p38-MAPK activity reflects the expression patterns of E2F1 and FOXM1 in both epirubicin sensitive and resistant MCF-7 breast cancer cells, providing a clue that p38 is involved in regulating E2F1 expression and epirubicin resistance. In agreement, results from studies using pharmacological inhibitors, siRNA knockdown and knockout MEFs revealed that p38 mediates the E2F1 induction by epirubicin and that its downstream kinase MK2 (MAPK-activated protein kinase 2; MAPKAPK2) is the intermediary of this induction. Furthermore, in vitro phosphorylation assays showed that MK2 can directly phosphorylate E2F1 at Ser-364. Although epirubicin treatment also affects other phosphorylation events, our transfection assays also demonstrated that E2F1 phosphorylation at Ser-364 participates in its induction by epirubicin.

Conclusions: We have also identified Ser-364 of E2F1 as a MK2 phosphorylation acceptor-site in response to epirubicin. Collectively, these findings underscore the importance of p38-MK2 signalling axis in the regulation of E2F1 and FOXM1 expression as well as drug sensitivity in response to epirubicin. Our findings highlight the important implications for therapeutic interventions as well as predicting chemotherapy treatment and sensitivity.

733 Common Breast Cancer Susceptibility Alleles in BRCA-positive and BRCA-negative Male Breast Cancer

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Background: Over the last 4 years, common low-penetrance breast cancer (BC) susceptibility alleles have been reported in a total of 24 loci, identified through GWAS or candidate gene approach. Interestingly, SNPs in these loci seem also to be associated with particular clinical-pathologic features of BC, such as hormonal receptors status, and *BRCA1/2* mutational status. Recently, an involvement of low-penetrance alleles in male BC (MBC) susceptibility has been suggested, however, whether these loci are associated with clinical-pathologic features of MBC or *BRCA1/2* mutational status is still largely unknown. Our aim was to evaluate the impact of 8 selected low-penetrance alleles in MBC susceptibility, and to assess associations between BC susceptibility alleles and clinical-pathologic features of MBC, including *BRCA1/2* mutational status.

Material and Methods: A case-control study was performed on a large MBC series collected in the first Italian multicentre study on MBC. A total of 395 MBC cases, including 46 *BRCA1/2* mutation carriers, together with their clinical-pathologic characteristics, and 847 male controls, including 124 unaffected *BRCA1/2* mutation carriers, were genotyped by allelic-discrimination real-time PCR with TaqMan probes at *FGFR2* rs2981582, *TNRC9* rs3803662, *MAP3K1* rs889312, *LSP1* rs3817198, 2q35 rs13387042, *ESR1* rs2046210, 5p12 rs10941679 and *CASP8* rs1045485.

Results: We found the greatest associations with overall MBC risk for SNPs at *FGFR2* (per allele OR, 1.21; 95% CI, 1.02–1.44), *TNRC9* (per allele OR, 1.50; 95% CI, 1.26–1.78) and *ESR1* (per allele OR, 1.67; 95% CI, 1.41–1.98). Statistically significant associations ($p < 0.05$) emerged between *FGFR2* and *TNRC9* minor alleles and *BRCA2*-negative MBCs, and between *ESR1* minor allele and *BRCA2*-positive MBCs. *ESR1* was indeed associated with PR−, HER2+, higher tumor grade and BC family history, whereas *FGFR2* and *TNRC9* were associated with PR+, HER2−, lower tumor grade and absence of BC family history.

Conclusions: Overall, based on a large multicentre series, our data support the hypothesis that common low-penetrance BC susceptibility alleles play a role in MBC susceptibility. Moreover, our results suggest that specific loci may be associated with distinct MBC subtypes and may act as genetic modifiers of *BRCA2* in men.

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734 Hepatitis C Viral Proteins Modulate Apoptosis and Inflammation Through Bid Protein

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Introduction: Hepatocellular carcinoma (HCC) is the 5th most common cancer and the 3rd cause of cancer related mortality worldwide. Viral infection by hepatitis C (HCV) or B (HBV) viruses is by far the most frequent etiology of HCC.

Because of dramatic lack of efficient therapies for HCC, prevention remains the utmost priority for hepatitis C patients. Among the multiple viral strategies to avoid elimination by the host immune response, HCV modulates the apoptotic response of hepatocytes by downregulating Bid, a BH3-only protein of the Bcl-2 family, rendering the cell resistant to the extrinsic apoptotic signalling and thus to elimination by cytotoxic T lymphocytes.

It has recently been shown that Bid also paricipates in the control of innate immune response. It interacts with NOD intracellular receptors, which are involved both in anti-microbial and anti-viral response. In view of the importance of the inflammatory context for tumorigenesis, the direct effect of HCV proteins on these innate immunity receptors are likely to be highly relevant to the initiation and progression of HCC.

Material and Methods: We have used an *in vivo* model of HCV transgenic mice (FL-N/35) and cellular models of primary human hepatocytes infected with HCV as well as HepaRG and Huh-7 cell lines.

Expression of Bid has been assessed by western blots and RTqPCR and its consequences by apoptosis assays. Stimulation of NOD and RIG-I pathways has been achieved by treatment with appropriate ligands and assessed by quantification of inflammatory cytokines.

Results and Discussion: HCV protein NS5A activates cellular calpains that degrade Bid. Interestingly, pharmacological inhibition of calpains restores