erlotinib exposure in vivo to those observed ex vivo in human hair treated with erlotinib.
Conclusion: We conclude that plucked human scalp hair represents an ideal minimally invasive surrogate tissue, with which to monitor drug response in patients receiving treatment with EGFR inhibitors.

PP 97

The relevant role of angiogenesis pathway in BRCA1/2 breast cancers

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Background: Germline mutations in the BRCA1 and BRCA2 genes are related with familial predisposition to breast cancer. Tumor angiogenesis and its important in breast cancer has been extensively investigated. The most important regulator of angiogenesis is the vascular endothelial growth factor (VEGF), up-regulated during hypoxia by hypoxia-inducible factor-1 (HIF-1α), a protein released during oxygen stress. Few is reported about angiogenesis in hereditary breast cancer, but it is known that there is an overexpression of HIF-1α in BRCA1 related cancers respect to sporadic cancers; and any evidence exists about correlation of VEGF, HIF-1α and microvessels formation in these tumors. Aim of this study is verified a differential expression of VEGF and HIF-1α and micro-vessels development, by CD31 marker, and a possible correlation of these markers in BRCA1/2 related cancers, compared with familial and sporadic breast cancers.
Materials and Methods: We investigated the expression of VEGF, HIF-1α and CD31 in 18 BRCA1, 7 BRCA2 mutated cancers and in 94 familial and 93 sporadic cancers, by immunohistochemistry.
Results: VEGF resulted more expressed in BRCA1-related cancers than in familial ($p < 0.01$) and in sporadic cancers ($p < 0.001$). However, VEGF expression was higher in familial than in sporadic group ($p < 0.001$). Also the MVD was significantly higher in BRCA1 cancers than familial and sporadic group ($p < 0.001$). Further higher microvascular density was associated with elevated VEGF expression. HIF-1α expression was more intensive in BRCA1 than in familial cancers ($p < 0.05$). In addition, from the analysis of BRCA2 cancers was clear that VEGF expression was higher in BRCA2 mutated than in sporadic cancers ($p < 0.01$). On the contrary the MVD was stronger in BRCA2 mutated than both familial and sporadic ($p < 0.01$). Further also HIF-1α showed a more intensive expression in BRCA2 mutated than both familial and sporadic cancers ($p < 0.001$).
Conclusion: Our results showed an increased expression of VEGF, CD31 and HIF-1α in hereditary cancers. These findings suggest that the angiogenesis plays a crucial role also in hereditary breast cancers.

PP 37

Antitumor activity and antioxidant status of berberine against Ehrlich ascites carcinoma in Swiss albino mice

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Background: Berberine has anti-tumor properties in various cancer cells including breast cancer but the exact mechanisms and in vivo effects are unclear. We investigated anti-cancer activity of berberine in vivo in Ehrlich ascites carcinoma (EAC) tumor.
Materials and Methods: 15×106 EAC cells were implanted intraperitoneally (i.p., ascitic tumor) in Swiss albino female mice. Fifty mice were divided into five groups and received drug for 14 days: group (1) saline, group (2) Cyclophosphamide (CP, 10 mg/kg, ip), group (3) berberine (6 mg/kg/d, ip), group (4) berberine (12 mg/kg/d, ip), group (5) berberine (18 mg/kg/d, ip). On day 15, blood samples were collected for hematological assessment of hemoglobin (Hb %), RBCs, WBCs and PCV. Ascitic fluid was also collected by making an incision in the abdominal region of mice. All mice were then sacrificed; sections from and liver were cut and homogenized for biochemical analysis measuring glutathione (GSH), malondialdehyde (MDA) catalase (CAT) and superoxide dismutase (SOD) activity. Also sections from solid tumor formed at peritoneal wall were removed for pathological examination after staining with (H & E).
Results: Berberine significantly inhibited tumor growth, cell viability in Ehrlich ascites tumor growth in vivo ($p < 0.001$). Histopathological examination of tumor cells in the treated group demonstrated signs of apoptosis with chromatin condensation and cell shrinkage. Decreased peritoneal angiogenesis showed the anti-angiogenic potential. Berberine at 12 mg/kg dose significantly increased in SOD and CAT activity ($p < 0.01$). GSH and TBARS were increased by 46 and 58% compared with control group ($p < 0.001$). Furthermore, berberine increased total RBCs, WBCs as well as Hb% significantly ($P < 0.05$) compared to CP.
Conclusion: Administration berberine inhibited the growth of Ehrlich ascites tumor. The results indicate that berberine exhibited significant antitumor and antioxidant activity in EAC-bearing mice.

PP 17

Monitoring phosphorylation of p70S6K1 kinase (Thr421/Ser424), a biological marker for mTOR activity, improves Nottingham histoprognostic grading of invasive breast carcinomas

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Background: Histological grade is one of the strongest prognostic factors in operable breast carcinomas (BC). 30−60% of BC are classified as histological Grade 2, which is associated with an intermediate risk of recurrence and is not informative for clinical decision making. The prognostic abilities of BC gene expression signatures such as the Genomic Grade Index (GGI), which can reclassify Grade 2 BC into 2 groups with high versus low risks of recurrence, are due mostly to the detection of proliferation activity. One of the strongest, yet simple and well-reproducible proliferation-associated prognostic factors is the mitotic activity index (MAI).
Materials and Methods: We have tested whether immunohistochemical assessment of MAI by monitoring phosphorylation of p70S6K1 (Thr421/Ser424) significantly impacts on the histopathological classification of Grade 2 BC. (1.) We validated the sensitivity of phospho-p70S6K1 Thr421/Ser424 (PP-S6K1) labeling in detecting & counting mitotic figures and also its usefulness for histoprognostic grading in a series of 144 BC biopsies; (2.) we investigated the correlation between PP-S6K1 MAI and the MAI determined by using the mitosis-specific marker phospho-Histone H3 Ser10 (PP-H3).
Results: PP-S6K1-labeled mitotic figures were easily seen and permitted a quick identification of the area of highest mitotic activity, even at low-power magnification. A statistically significant correlation was found between the mitotic counts obtained by using PP-S6K1 and those assessed by either standard Hematoxylin & Eosin [H & E] ($r = 0.680$) or PP-H3 staining ($r = 0.855$). PP-S6K1 MAI correlated also with tumor proliferative activity as measured with the Ki-67 labeling index ($r = 0.628$). Average mitotic counts were significantly higher when using labeling with PP-S6K1 (range = 0–141, mea n = 12) or PP-H3 (0–146, 19) than with the standard H&E protocol (0−60, 5). Importantly, when the global histoprognostic score of the Nottingham Grading System was re-evaluated on the basis of PP-S6K1 staining, there was a statistically significant shift from Grade 1 to Grade 2 in 7 cases, and from Grade 2 to Grade 3 in 17 cases. Indeed, PP-S6K1 was as efficient as PP-H3 at reclassifying BC patients with H & E-determined Grade 2 tumors.
Conclusion: Our findings reveal that immunolabeling with PP-S6K1 − a biological marker for activity of the mTOR signaling pathway − constitutes a simple and reliable method for quantifying proliferative potential that significantly improves Nottingham histoprognostic grading of BC.

PP 22

Analysis of HER-3, insulin-growth factor-1 (IGF-1), nuclear factor k-B (NF-kB) and epidermal growth factor receptor (EGFR) gene copy number (GCN) in the prediction of clinical outcome for K-RAS wild type colorectal cancer patients receiving irinotecan–cetuximab

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Background: A large proportion of colorectal cancer patients does not benefit from the use of anti-EGFR treatment although in the absence of a mutation of the K-RAS gene. Preliminary observations suggested that HER-3, IGF-1, NF-kB and EGFR GCN might identify patients not likely to benefit from anti-EGFR therapy. We tested the interaction between HER-3, IGF-1, NF-KB, EGFR GCN and K-RAS mutational analysis to verify the relative ability of these variables to identify a sub-group of patients more likely to benefit from EGFR-targeted treatment among those harbouring a K-RAS wild type status.
Materials and Methods: We retrospectively collected tumours from 168 patients with metastatic colorectal cancer patients treated with irinotecan-cetuximab. KRAS was assessed with direct sequencing, EGFR amplification was assessed by chromogenic in situ hybridization and HER-3, IGF-1 and NF-kB were assessed by immunoistochemistry.
Results: In patients with K-RAS wild type tumours, the following molecular factors resulted independently associated with response rate: HER-3 (OR = 4.6, 95% CI: 1.8–13.6, $p = 0.02$), IGF-1 (OR = 4.2, 95% CI: 2–10.2, $p = 0.003$) and EGFR GCN (OR = 4.1, 95% CI: 1.9–26.2, $p = 0.04$). These factors also independently correlated with overall survival as follows: HER-3 (HR = 0.4, 95% CI: 0.28–0.85, $p = 0.008$), IGF-1 (HR = 0.47, 95% CI: 0.24–0.76, $p < 0.0001$) and EGFR GCN (HR = 0.59, 95% CI: 0.22–0.89, $p = 0.04$).
Conclusion: We believe that our data may help further composing the molecular mosaic of EGFR resistant tumours. The role of HER-3, IGF-1 and CISH EGFR GCN should be prospectively validated in clinical trials investigating anti-EGFR treatment strategies in colorectal cancer patients.