whereas S12 cells, which derived from the W12 line, contain HPV DNA as integrated copies. E2 gene region was screened by using PCR with three separate primers covering the whole genome. Cells where irradiated with singles doses of 0 Gy, 1 Gy, 2 Gy, 3 Gy, 4 Gy, 5 Gy and 7 Gy. Clonogenic survival was analyzed by using the 96-well in vitro test. Survival fraction and survival curves where calculated using Sigma Plot 8.0. At least three experiments where performed for each dose point.
Results: The E2 gene of the S12 cells (passage 88–103) was disrupted in the E2C region. The W12 cells (passage 8−14) with an intact E2 gene showed a higher radiosensitivity with a radiation enhancement factor of 1.5 (4 Gy).
Conclusion: HPV 16 positive W12 cells with an intact E2 gene showed a higher intrinsic radiosensitivity compared to those with an disrupted E2 gene. About 30% of patients with cervical cancer have tumors with an intact E2 gene. Our experiments indicate a better response to radiation treatment might be factor for their better prognoses.

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Gossypol activates the SAPK/JNK pathway and enhances radiation-induced apoptosis

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Background: Overexpression of anti-apoptotic members of the Bcl-2 family has been associated with treatment resistance and poor outcome. Gossypol (GP) is a small molecule inhibitor of Bcl-XL and Bcl-2 and induces apoptosis in a wide range of tumor cell lines. Here, we tested the effect of GP on radiation-induced cell death in a panel of human head and neck cancer (HNSCC) and leukemic cell lines Because activation of the SAPK/JNK pathway is important for apoptosis induction by other stimuli, we also investigated the role of this signaling cascade in GP-induced apoptosis.
Material and Methods: Four types of human HNSCC (UM-SCC-11B, UM-SCC-22A, UM-SCC-14C, VU-SCC-OE) and 2 leukemic cell lines (Jurkat T, U937) were treated with increasing doses of GP, radiation and the combination. Apoptosis was quantified by FACS analysis; SAPK/JNK activity was measured by Western blot; isobolographic analysis was performed to characterize the interaction between radiation and GP.
Results: In all cell lines tested, GP induces apoptosis in a time- and dose-dependent fashion, with ED50 values in the uM range. Like radiation, GP rapidly activates SAPK/JNK which can be blocked by the kinase inhibitor SP600125. To demonstrate the critical role of SAPK/JNK activation in GP-induced apoptosis, U937 cells stably expressing the dominant negative mutant of c-Jun, TAM-67, were used. In these U937-TAM-67 cells both radiation- and GP-induced apoptosis was significantly reduced as compared to vector-only controls. By combining radiation and GP, in particular radiation given 24 hours before GP, apoptosis was strongly enhanced. Isobolographic analysis revealed a synergistic interaction between both stimuli.
Conclusion: GP strongly enhances radiation-induced apoptosis in human HNSCC and leukemic cells. Our studies also indicate a requirement of the SAPK/JNK pathway in this response. This type of apoptosis modulation may lead to the development of new effective combination therapies.

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HER2 polymorphism is associated with gastric cancer risk

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Introduction: Gastric adenocarcinoma (GC) is a major public health problem worldwide. In Portugal, GC represents a sixth of all cancer related deaths, with twice the average mortality of European Union. Several host genetic variations have been regarded as potential risk markers for this neoplasia. The Human Epidermal growth factor Receptor-2 (HER2) plays an important role in cell differentiation and proliferation, being associated with cancer evolution. A single-nucleotide polymorphism in the HER2 gene (Ile – Val) was described. The aim of this study was to evaluate the role of this polymorphism in the development of GC within a southern European population.
Materials and Methods: We conducted a case-control study on 484 individuals, including 162 patients with histological confirmed GC and 322 healthy blood donors from the same geographical area as the cases. DNA extracted from peripheral blood was submitted to Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP), in order to identify the possible HER2 genotypes; Ile/Ile, Ile/Val and Val/Val. The restriction fragments were analyzed in a 3% agarose gel, stained with ethidium bromide.
Results: We found that the frequency of the Ile/Val genotype was higher in gastric cases (36.4%) than in controls (30.1%), and the same was observed with the Val/Val genotype (4.9% and 2.2%, respectively). A twofold increase in the risk of gastric cancer was found among carriers of Ile/Val and Val/Val genotypes (OR adjusted to age = 2.04; 95% CI: 1.18−3.52; p = 0.011). This risk was even higher when we analyzed only female individuals (adjusted OR = 3.18; 95% CI: 1.44−7.00; p = 0.004). Moreover, we observed that the median time-to-onset of gastric cancer was shorter in the patients carrying the Val allele (63.6 vs 71.6 months for all patients; p = 0.011; and 64.9 vs 78.9 months for women; p = 0.004).
Conclusions: Our results indicate an association between the presence of the Val allele in the HER2 polymorphism and the risk of gastric cancer. Studies hypothesize that the presence of this allele has been implicated in the formation of active HER2 receptors, leading to enhanced signal transduction activation, which may therefore trigger carcinogenesis. In this study, the risk of gastric cancer was even higher in women, and an association between HER2 and estrogen has been extensively studied. Further studies are needed to elucidate this association.

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Expression of intratumoral lactate dehydrogenase 5 (LDH5) and expression of biomarkers for angiogenesis and hypoxia are linked in patients with colorectal cancer (CRC)

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Background: Results from two Phase III randomized clinical trials in 1st line (CONFIRM 1) and 2nd line (CONFIRM 2) metastatic CRC indicated that PTK787/ZK222584 (PTK/ZK), an oral tyrosine kinase inhibitor (TKI) which blocks all known VEGF receptors (VEGFR), demonstrated greatest efficacy in patients (pts) with high baseline serum lactate dehydrogenase (LDH) levels (>1.5 ULN). To better understand the relationship between angiogenesis, tumor hypoxia and acidosis, we investigated whether CRC pts with high levels of tumor LDH5 would also have increased expression of proteins linked to tumor-cell hypoxia [hypoxia inducible factors: HIF-1 alpha (a), and -2a] dehydrogenase kinase (PDHK) and angiogenesis [VEGFA; phosphorylated VEGFR2 (pKDR)], as well as acidity [carbonic anhydrase 9 (CA9)]. We also assessed vessel density (VD).
Methods: Using a nominal scoring system, we will conduct an immunohistochemical analysis of tissue-sections of primary or metastatic tumor taken from over 100 participants of the CONFIRM 1 and 2 trials. A measure of association between the scores for protein-expression will be estimated by the phi-coefficient (correlation coefficient) and assessed by means of p-values from pairwise Fisher's exact test (two-sided).
Results: An earlier analysis of 42 tissue samples revealed associations between the expression levels of LDH5 and the following proteins: pKDR (Phi = 0.53; p < 0.001), VEGF (Phi = 0.41; p = 0.006), VD (Phi = 0.34; p = 0.052), HIF-1alpha (Phi = 0.56; p < 0.001), and PDHK (Phi = 0.58; p = 0.0014). HIF-1a was associated with pKDR (Phi = 0.38; p = 0.027), VD (Phi = 0.34; p = 0.045), and VEGFA (Phi = 0.33; p = 0.067) expression. VEGFA was associated with PDHK (Phi = 0.52; p = 0.035). These results will be updated using data derived from over 100 samples.
Conclusions: Tumor samples from mCRC patients with high levels of intratumoral protein expression of LDH5 also demonstrated elevated HIF-1a, pKDR, VEGFA, PDHK expression and VD. The results suggested a link between tumor hypoxia and angiogenesis, and demonstrate elevated LDH protein expression may serve as a surrogate marker for an activated