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Molecular alterations of KIT and PDGFR-alpha; in GISTs – A Portuguese experience

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Background: GISTs are rare primary mesenchymal tumours of the gastrointestinal system. Detection of Kit overexpression by immunohistochemistry is a hallmark of GIST diagnosis. Kit is a type III receptor tyrosine kinase, belonging to the PDGFR family. Kit overexpression usually results from activating mutations in the extracellular domain (exon 9), juxtamembrane domain (exon 11), and intracellular kinase domains (exons 13 and 17) of *KIT* oncogene. Approximately 80% of *KIT* mutations occur in exon 11, and are predictive of high sensitivity response to tyrosine kinase receptor inhibitor, Imatinib®. *KIT* wild-type GIST cases may harbour alternative activating mutations of *PDGFR-α,* hotspot region (exons 12, 14 and 18), in a mutually exclusive way. The frequency of *KIT* mutations ranges from 20 to 80% of GIST reported cases. In Portugal, the incidence of *KIT* and *PDGFR-α* mutations in GISTs is virtually unknown. The aim of this work was to assess the frequency of *KIT* and *PDGFR-α* oncogenes mutations in a Portuguese series of GISTs.
Material and Methods: Eighty-one formalin-fixed and paraffin-embedded *bonafide* primary previously untreated GISTs were selected after clinical-pathological review. Mutation analysis of *KIT* exon 11, as well as of *PDGFR-α* (exon 12, 14 and 18) was done by direct sequencing. Analysis of *KIT*, exons 9, 13 and 17 was done by PCR-SSCP, followed by direct sequencing.
Results: Of the 81 GISTs, 38 (47.0%) contained mutations in exon 11, 4 (4.9%) in exon 9, and 1 (1.3%) in each exon 13 and 17 of *KIT*. Mutations in exon 11 included deletions (44.7%), duplications (2.6%), base substitutions (36.8%), and mixed mutations (15.8%), i.e. deletions together with either insertions or base substitutions. All but one mutation in exon 9 were the same duplication (502dup503). All deletions and duplications were in-frame. Alterations in exon 13 and 17 were silent mutations. Of *KIT* mutation negative cases, *PDGFR-α* mutations were present in 3/18 (16.7%) in exon 12, 5/29 (17.2%) in exon 18, and none in exon 14 [0/15 (0%)]. Mutations in exon 12 and 18 included deletions (25%) and base substitutions (75%). Twenty (24.7%) GISTs were "wild-type" for both oncogenes. In eight *KIT* wild-type bearing GISTs, *PDGFR-α* was not possible due to available material. No correlation was found between mutations of both *KIT* and *PDGFR-α* oncogenes and clinical-pathological features (e.g. risk group, tumour size, and location); lack of *KIT* mutation was statistically associated ($p < 0.0197$) with epithelioid GISTs.
Conclusions: The incidence of mutually exclusive *KIT* (54.3%) and *PDGFR-α* (10.9%) mutations in this Portuguese GISTs series fits with published series, namely of South European Countries. Epithelioid GISTs usually do not harbour *KIT* mutations. As reported, the great majority of *KIT* mutations 38/44 (86.4%) are located in exon 11, indicating a favourable response to Imatinib-based therapy.

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Time dependent effects of Gefitinib on ABCG2: modulation of CPT-11 efficacy in colon cancer cell lines

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Recent data indicate that ABCG2 displays a high-affinity interaction with several tyrosine kinase receptor inhibitors, including Gefitinib, which seem to be responsive of an increase sensitivity to camptothecins. These data suggest a crucial role of this pump in multidrug therapy, with the combination of EGFR inhibitors with camptothecins. Our previous study concerning the possibility to combine gefitinib and SN-38 (the active metabolite of CPT-11) put in evidence that the two drugs were synergic or antagonist in function of the schedule utilised; in fact, when SN-38 was given before gefitinib, they sinergically increased cell growth inhibition conversely, in the opposite schedule they were antagonist.
In order to explain the opposite effects of the two sequential schedule of gefitinib plus SN-38 and to investigate the involvement of ABCG2 in these phenomena, we analysed the capability of the TK inhibitor to modulate ABCG2 expression in function of time, by western blot analysis, and as a consequence, the modulation of SN-38 intra-cell accumulation after the cell pre-exposure to gefitinib, by HPLC analysis. Moreover, we analysed the capability of gefitinib to modulate SN-38 activity, as cell cycle perturbation, after short gefitinib exposure (1hour) and in function of drug concentration, by flow cytometry. In this study, we utilised gefitinib at a sub-active concentration (IC30) and SN-38 at the IC50 concentration in two colon cancer cell lines, LoVo and HT-29.
Our results showed that ABCG2 expression was modulate by gefitinib only after prolonged exposure, with a stimulation from 5 to 14 days conversely, at short time (from 15 min to 3 days) it was not affected. These evidences suggest to determine SN-38 accumulation after 5 days gefitinib exposure and it was strongly reduced, justifying the antagonism between the two drugs on cell growth. Moreover, the analysis of cell cycle modulation by short time gefitinib exposure (1day) showed that the cell accumulation in S-phase, when the two drugs were given together or gefitinib before SN-38, was reduced as respect the SN-38 alone and with a gefitinib concentration-dependency conversely, the synergic schedule, SN-38 followed by gefitinib showed an increased S-phase accumulation. In this report and for the first time, we provide evidences that gefitinb could act as an ABCG2 inhibitor, when given for short time, as already reported by other authors, and as a stimulator of this pump after prolonged exposure These preliminary results confirmed that the interaction between gefitinib and ABCG2, in function of time exposure, could be a very relevant factor necessary to consider when a multidrugs therapy is planned.

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Sphingolipids enhance expression of the multidrug resistance phenotype in human breast cancer cells

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Background: Although overexpression of P-glycoprotein (P-gp) by the multidrug resistance gene (MDR1) is one of the most consistent alterations associated with cancer cell resistance to chemotherapy, lipids such as ceramide also affect response to chemotherapy. For example, many natural-product anticancer agents enhance cellular levels of ceramide, a proapoptotic sphingolipid. Because high levels of the ceramide metabolite glucosylceramide (GC) often coincide with elevated P-gp, we hypothesized that ceramide and GC might influence expression of the multidrug resistance phenotype.
Materials and Methods: Three wild-type human breast cancer cell lines (MDA-MB-435, MDA-MB-231, T47D) were exposed for several days or several passages to short-chain, cell-permeable ceramide (C8-cer) or glucosylceramide (C8-GC). MDR1 expression (mRNA) was determined by real-time RT-PCR (beta-actin internal control); expression of P-gp was determined by Western blot. Cell sensitivity to chemotherapy was evaluated by viability assays in 96-well plates, and cell efflux capacity was assessed by using rhodamine-123.
Results: Short-term (3-day) exposure to C8-cer (5.0 ug/ml media, 11.7 uM) enhanced MDR1 mRNA levels by 3-fold and 5-fold in T47D and MDA-MB-435 cells, respectively. Growth of MDA-MB-231 cells with C8-cer for extended periods enhanced MDR1 expression by 45-fold and 370-fold at passages 12 and 22, respectively. This was accompanied by an increase in P-gp levels, enhanced rhodamine efflux, and a decrease in cellular sensitivity to chemotherapy (doxorubicin, paclitaxel). Short-term (3-day) treatment with C8-GC (10 ug/ml media) increased MDR1 expression by 4-fold in MDA-MB-231 cells as compared with glycolipid-naïve controls. Exposure of cells to octanoic acid, a C8-cer hydrolysis product, or to oleic acid (18:1) did not alter MDR1 expression.
Conclusions: Here we show for the first time that ceramide and glycolipids upregulate MDR1 expression in breast cancer cells. This suggests that ceramide's role as a messenger of cytotoxic response to chemotherapy is linked to the multidrug resistance pathway. Targeting GC synthesis might circumvent resistance to natural product chemotherapy.

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THPC11PtCl2: a novel platinum compound that overcomes cisplatin resistance by inducing a different mechanism of apoptosis

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Background: Despite cisplatin (CDDP)-based chemotherapy are curative in testicular germ cell tumors (TGCT), the development of platinum drugs with improved antitumoral activity continues to be a productive field of research. Particularly with regard to circumvent CDDP resistance we designed a new platinum derivative THPC11PtCl2, consisting of cisplatin linked to a tetrahydropyrane via an aliphatic C11-spacer. The purpose of the present work was to compare the cytotoxic potential of this drug with CDDP using the CDDP-sensitive TGCT-cell line H12.1 and the CDDP-resistant TGCT-cell line 1411HP.