activated in cancer and whether its modulation may have an effect on cancer.
Materials and Methods: LysRS status was analyzed by using protein fractionation assays as described by Yannay-Cohen et al. We used Hela cells and H460 cells overexpressing EGFR generously provided by G. Batist (Mcgill U. Montreal) to study possible relationship to the EGFR pathway. Hint constructs were cotransfected with wildtype or mutant LYSRS encoding constructs to A549 lung cancer cells.
Results: We checked EGFR effects on this pathway − EGF activation of HELA cells resulted in movement of LysRS to smaller protein fractions implying release of LysRS from the multisynthetase comple. Inhibition with Gefitinib of EGFR in a NSCLCA line resulted in the opposite effect. MAPK inhibition resulted in the disappearance of LysRS from smaller protein fractions in a cell line with overactivated MAPK pathway, implying that the presence of LysRS in smaller complexes can be manipulated by MAPK inhibition. Transfection of S207DLys RS pseudophosphorylated mimic with HINT1 prevented HINT1 activity as a tumour suppressor in a colony assay of A549 cells.
Conclusion: Our results suggest that the lysRS-AP4A pathway is activated in cancer. Activation of such a pathway in cancer might have distinct effects as shown in the case of HINT1tumour suppression. This is the first demonstration of the activity of this pathway in cancer. Other signal transduction pathway operating according to the principles of the depot theory may be aberrantly overactivated in cancer. Further research is needed to delineate the precise biological significance of this pathway in different cancers.

1044 POSTER

Acquisition of P-glycoprotein Overexpression in Sensitive Tumour Cells

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Background: The overexpression of P-glycoprotein (Pgp/ABCB1), a drug efflux pump, promotes multidrug resistance (MDR) which prevents the successful of clinical cancer treatment. In addition, the overexpression of survivin and XIAP (inhibitors of apoptosis proteins − IAPs) may contribute to MDR phenotype in association with Pgp expression. Recently, it has been reported that Pgp expression can be acquired through transfer of membrane microparticles. Based on this, the aim of this study was establish an *in vitro* model to intercellular transfer of Pgp, and analyze the consequence of this phenomenon on IAPs expression in tumour cells.
Materials and Methods: K562–Lucena cell line (Pgp-positive cells derived from chronic myeloid leukemia) was co-cultivated with MCF7 and A549 cell lines (Pgp- negative cells derived from breast adenocarcinoma and lung carcinoma, respectively) for 24h and 48h. After co-culture, Pgp expression of recipient cells (MCF7 and A549) was assessed by immunostaining and immunofluorescence. Survivin and XIAP expression were analyzed by Western blot and qRT-PCR.
Results: The efficiency of our *in vitro* model to intercellular Pgp transfer was confirmed by flow cytometry, in which we observed high levels of Pgp expression in recipient MCF7 and A549 cell lines after 24h and 48h of co-culture. Furthermore, we observed Pgp expression on plasma membrane of recipient MCF7 and A549 cells after both times by immunofluorescence microscopy, which also revealed clusters of Pgp, possibly suggesting the identification of microparticles. Additionally, we also evaluated survivin and XIAP expression after acquisition of Pgp expression in MCF7 and A549 cell lines. We observed an increase on survivin and XIAP mRNA and protein expression in Pgp-positive A549 cells at 24h and 48h. However, we could not observe changes on survivin and XIAP mRNA and protein expression in Pgp-positive MCF7 cells. These data suggest that Pgp contribution on IAPs expression is cell line-dependent.
Conclusions: Taken together, these data demonstrate that sensitive cell lines acquired expression of MDR proteins when in contact with resistant cells. Besides that, these findings contribute to our knowledge for the emergence of MDR in tumour cells and could be helpful for new treatment approaches.
Acknowledgements: INCT para Controle do Câncer, CNPq 573806/2008−0; FAPERJ EE26/170.026/2008.

1045 POSTER

Pharmacological Targeting Chaperone Activity and Chaperone Expression Enables to Sensitize Human Tumours to Hyperthermia and Radiotherapy

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Background: Ionizing radiation and hyperthermia are therapeutic modalities in fight against cancer. Because multiple heat shock protein 90 (Hsp90)-dependent pathways ensure tumour cell survival, pharmacological inhibitors of the Hsp90 chaperone activity, e.g. 17-N-allilamino-17-demethoxygeldanamycin (17AAG), may be synergistic with antitumour effects of hyperthermia or radiation. The problem is that 17AAG activates the heat shock transcription factor 1 (HSF1) thus inducing cytoprotective chaperones Hsp70 and Hsp27 which enhance thermo- and radioresistance of the 17AAG-treated tumour cells. Here we combined 17AAG with inhibitors of the HSF1-mediated Hsp induction to sensitize human tumour cells to mild hyperthermia and clinically relevant doses of radiation.
Materials and Methods: Hyperthermia (42–43 C, 60 min) or gamma-irradiation (2−6 Gy) were used as mono-treatments or with 17AAG and inhibitors of the Hsp induction such as quercetin, triptolide or NZ28 to kill MCF-7 and HeLa cells derived from human breast or cervical tumours. The cytotoxicity was determined in fluorescence staining and clonogenic assay. The protein levels were analyzed by immunoblotting. The 17AAG-induced inhibition of Hsp90 chaperone activity was assessed on retardation of the chaperone-dependent reactivation of luciferase in the heat-stressed transfectants.
Results: It was found that 20−100 nM 17AAG enhanced apoptosis and impaired clonogenicity in the cancer cells subjected to hyperthermia or low doses (2−4 Gy) of gamma-radiation. This enhancement of cytotoxicity correlated with a degree of the Hsp90 chaperone activity. As biomarkers of the Hsp90 activity inhibition, the specific depletion of Akt and Raf-1 and upregulation of Hsp70 and Hsp27 were revealed in the samples of 17AAG-treated cells. Addition of either 10−30 uM quercetin or 2−5 nM triptolide, or 0.3−1 uM NZ28 fully prevented the Hsp accumulation in the 17AAG-treated cancer cells and rendered them much more sensitive to hyperthermia and radiation; the powerful sensitizing effects of extremely low concentrations of triptolide seemed particularly impressive.
Conclusion: The synergism in cytotoxicity under combining of hyperthermia or gamma-radiation with 17AAG and triptolide appears to be due to (i) blockade of the Hsp90-dependent antiapoptotic pathways, and (ii) blockade of the 17AAG-induced upregulation of cytoprotective Hsp70 and Hsp27, while the target cells undergo cytotoxic treatments.

1046 POSTER

Expression of NF-kB Transcription Factor, C-erbB2, Estrogen and Progesterone Receptors in Tumours of Patients With Breast Cancer

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Background: The aim of present study was to study expression of NF-kB (p50/p65), c-erbB2, ER and PR in breast tumours and analyse correlation between these markers.
Methods: We selected 41 patients with locally advanced breast cancer who had not been treated with radio- or chemotherapy. The I stage was diagnosed for 4, II − for 27, III − for 6, IV − for 3 patients, 3 patients had "x" stage at that moment. All tumours belong to invasive ductal carcinoma with different tumour grade.
NF-kB, c-erbB2, ER and PR expression was determined by immunohistochemistry. For staining interpretation the H-score method was evaluated. The score is obtained by the formula:
3×% of strongly staining + 2×% of moderately staining +% of weakly staining. Expression level of markers was graded as weak (H-score 0−49), moderate (50−99) or strong (100−300).
Results: The association between ER and PR expression and grade was defined. G3 tumours had lower expression of receptors (H_{ERmean} = 77, H_{PRmean} = 23) in comparison to G2 (H_{ERmean} = 89, H_{PRmean} = 75) and G1 (H_{ERmean} = 143, H_{PRmean} = 95).
We had formed three groups of patients. Patients from first group had high expression of p50 and p65, second- high expression of p50, third- low expression of subunits. The relation between expression of NF-kB and other markers was evaluated. In the first group ER, PR and c-erbB2 expression was low (H_{ERmean} = 39, H_{PRmean} = 37, $H_{c-erbB2mean}$ = 34), in the second- moderate (H_{ERmean} = 73, H_{PRmean} = 47, $H_{c-erbB2mean}$ = 98), in the third- high (H_{ERmean} = 149, H_{PRmean} = 99, $H_{c-erbB2mean}$ = 123).
Also, NF-kB expression in different molecular types of breast tumours was analyzed. Breast tumours are sub-divided on Luminal (high/moderate ER, moderate/low c-erbB2), HER2 (low ER, high c-erbB2), Luminal-HER2 hybrid (high/moderate ER, high c-erbB2), and basal-like (low ER and c-erbB2) types. Our data show the highest p65 expression in basal-like type ($H_{p65mean}$ = 75), moderate in luminal ($H_{p65mean}$ = 43) and HER2 ($H_{p65mean}$ = 44) type, and low in Luminal-HER2 hybrid ($H_{p65mean}$ = 26) type.
Conclusion: As a result of researches was defined that G3 tumours have lower ER and PR expression in comparison to G2 and G1 tumours. p50 and p65 levels were found to be changed in dependence from expression of each marker (ER, PR, c-erbB2) and also from tumour's type (Lumilal, Her2, hybrid, basal-like). So, our findings are the reason for further study this characteristics, because the results of such investigations may be useful for search of chemotherapeutic scheme, and for prognosis of disease course.