318 POSTER

Changes in the transcription regulation mediated by FUS-CHOP gene in a myxoid liposarcoma cell line resistant to trabectedin

S. Uboldi[1], S. Bernasconi[1], M. Romano[1], S. Marchini[1], S. Di Giandomenico[1], G. Damia[1], M. Ganzinelli[1], C.M. Galmarini[2], E. Erba[1], M. D'Incalci[1]. [1]*Mario Negri Institute, Oncology, Milan, Italy;* [2]*PharmaMar, Cell Biology, Madrid, Spain*

Background: Trabectedin (T), a marine alkaloid, has shown striking antitumor activity in a variety of preclinical models. Clinical investigations have shown that T is effective in myxoid liposarcomas (MLS). In this disease, characterized by the chimeric protein FUS-CHOP, the drug is extremely active (a clinical response rate in 50% of patients). In order to investigate the mechanisms of resistance, a trabectedin resistant cell line, 402−91/ET, derived from MLS 402-91 cells, was developed.
Material and Methods: 402-91/ET cell line was made by a stepwise increase in drug concentration. Drug sensitivity was measured by colony assay, MDR related proteins by FACS analysis, the XPG by PCR and Western Blot, the binding of FUS-CHOP by Chip analysis.
Results: 402-91/ET cell line shows 10 fold stable resistance to trabectedin. No differences were found in MDR related proteins and in uptake/efflux of T between the parental and resistant cells. The cross resistance to Melphalan and trabectedin was reverted by GSH depletion. The collateral sensitivity of 402-91/ET cells to UV was related to the lack of XPG gene; the transfection of resistant cell line with an expression vector encoding the XPG cDNA reversed the UV hypersensitivity but not the resistance to T. 402−91/ET cell line showed a collateral sensitivity to temozolomide due to methylation of MGMT promoter. In 402−91 cells FUS-CHOP chimera, bound to PTX3 and FN1 promoters, was detached by trabectedin treatment whereas in 402−91/ET cells it was not bound.
Conclusion: We developed for the first time a stable model of MLS cell line resistant to trabectedin. The resistance mechanism of 402−91/ET to the drug is probably related to changes in the transcription regulation mediated by FUS-CHOP chimera. Studies are ongoing to elucidate the molecular mechanism by which this process occurs.

319 POSTER

Tumour hypoxia and ALDH expression may contribute to reduced response to therapeutic agents in HNSCC cell lines

M. Zimmermann[1], C. Box[1], P. Rhys-Evans[2], K.J. Harrington[3], S.A. Eccles[1]. [1]*The Institute of Cancer Research, Cancer Therapeutics, Sutton Surrey, United Kingdom;* [2]*The Royal Marsden Hospital, The Royal Marsden Hospital, London, United Kingdom;* [3]*The Institute of Cancer Research, Cell & Molecular Biology, London, United Kingdom*

Background: Despite a good initial response of head and neck squamous cell carcinomas (HNSCC) to conventional treatment (radiotherapy and chemotherapy), relapse is common and survival rates remain poor.
Responses to therapy can be affected by the tumour microenvironment (Fig.1). However, standard 2D monolayer cultures used to assay tumour sensitivity to drugs lack microenvironmental complexity and may be poor predictors of *in vivo* efficacy. For example, hypoxia, common in advanced solid tumours, is associated with increased invasion, metastasis, reduced drug efficacy and poor prognosis in HNSCC yet is not reflected in standard 2D cultures. In addition, 3D culture and hypoxic conditions may preferentially support cancer stem-like cells (CSCs), postulated to play a role in therapy-resistant recurrences, and to contribute to treatment failure and disease progression.

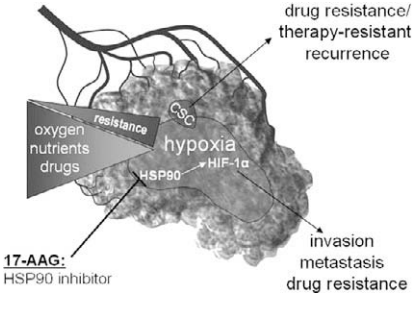

Fig.1

This study aims to determine the effects of hypoxia and 3D culture on CSC frequency and response to targeted therapies in HNSCC.
Material and Methods: Spheroids from HN4 (laryngeal SCC) and PJ41 (oral SCC) were generated using non-adherent culture conditions. CSCs were identified by flow cytometry using the "side-population" (SP) assay (Hoechst 33342 exclusion) or via expression of aldehyde dehydrogenase (ALDH) using the ALDEFLUOR assay.
Results: A SP that was up to 4-fold higher in 3D cultures than 2D was demonstrated in HN4 cells, suggesting the presence of potential CSCs. Additionally, flow cytometric-sorted $ALDH^{high}$ cells from PJ41 cells showed enhanced colony forming ability (CFA) compared to $ALDH^{low}$ cells (~3-fold) and were more resistant to 17-AAG (HSP90 inhibitor) in normoxia and hypoxia. The CFA of $ALDH^{high}$ and $ALDH^{low}$ cells was increased in hypoxia (up to 3-fold) compared to normoxia. In addition, preliminary studies showed that cells surviving 17-AAG treatment have an increased proliferation rate and a 2-fold greater CFA in hypoxia than in normoxia.
Conclusion: In summary, I have used two different flow cytometry-based assays to identify subpopulations of HNSCC cells which exhibit characteristics of CSCs and are more resistant to drug treatment. These warrant further investigation as causes of treatment failure in HNSCC patients.

320 POSTER

Enhancement of 5 fluorouracil (5FU)-mediated cytotoxicity in human gastric cancer (GC) cells by the phosphatidylinositol 3-kinase (PI3K) inhibitor, PI103, via thymidylate synthase (TS) inhibition

B. Bhattacharya[1], M. Akram[1], I. Balasubramanian[1], R. Soong[1]. [1]*National University of Singapore, Cancer Science Institute, Singapore, Singapore*

Background: GC is the second most lethal cancer worldwide. 5FU inhibits TS and is used in many malignancies including GC. However, low response rates and drug resistance hinders treatment. PI3K pathway is involved in cell survival and drug resistance. The pathway is frequently activated in GC (~40%) as a result of multiple aberrations. PI103 is a potent class I PI3K inhibitor. We hypothesized that inhibition of PI3K pathway in *PI3K* mutant GC will enhance the cytotoxicity of 5FU, at least in part, due to increase in apoptosis.
Methods: 5 GC cell lines were tested for *PIK3CA*, *KRAS* and *BRAF* mutations by sequencing. The IC50 of 5FU and PI103 were measured in GC cells at 72 h using an MTS assay. 5-FU was combined with PI103 at a fixed ratio of their IC50 continuously for 72h and the data analysed using the median effect equation of Chou and Talalay. Protein expression was carried out using immunoblotting and apoptosis measured using the cell death Elisa kit® kit (Roche). Cell cycle analysis was carried out by propidium iodide/RNase staining.
Results: Mutations were observed in *PIK3CA*, *KRAS* in 3 (60%) and 1 (20%) of the 5 cell lines respectively. The sensitivity of GC cells to PI103 or 5FU was independent of *PIK3CA* status. In *PIK3CA* mutant AGS, HGC27 and IM95 cells, PI103 was highly synergistic when combined with 5FU with a combination index (CI) at fraction unaffected (fu) 0.5 = 0.7, 0.2 and 0.5 respectively. The synergy was associated with decrease in TS protein level by 3× IC50 concentration of PI103 at 24 h. The combination also led to a marked increase in apoptosis compared to the single agents and maintained 5FU-mediated S phase arrest at 24 h, consistent with synergy. In contrast, in NUGC4 and MKN45 cells in which the PI3K pathway appears less deregulated, the combination led to antagonism or additivity (CI at fu 0.5 = 5.7, 1.2 respectively). 3× IC50 concentration of PI103 did not appear to inhibit TS at 24 h in NUGC4 cells and no increase in apoptosis above control was observed in response to the combination, consistent with antagonism. Cell cycle analysis demonstrated abrogation of 5FU-mediated S phase arrest in the presence of PI103 in NUGC4 cells at 24 h. Interestingly, reduction of TS protein by PI103 was observed in *PIK3CA* wild-type MKN45 cells at 24 h in which the combination is additive. Nonetheless, the additivity was supported by the effect of both compounds in combination on cell cycle distribution (G1 and late S phase arrest) and greater apoptosis than single agents at 24 h. Preliminary mechanistic studies in HGC27 (synergistic) and NUGC4 (antagonistic) cells revealed no apparent effect on the TS ternary complex by the combination at 24 h, suggesting inhibition of TS as a determinant of synergy.
Conclusion: These data suggest that inhibition of TS by PI103 may lead to at least additivity in combination with 5 FU in GC. However, synergy may be expected only in cells with mutant *PIK3CA*. Current work is ongoing to study the effects of scheduling the combination, other TS inhibitors, and deconvoluting the mechanisms of the synergy.