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Antitumor activity of brostallicin on human prostatic cancer cells: fundamental role of combination with hypomethylating agents

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Brostallicin preclinical antitumor activity depends on the intracellular levels of glutathione (GSH) and/or glutathione-S-transferase (GST). Among the GST isoenzymes the pi class is the stronger activator of brostallicin.
Almost 90% of human prostatic adenocarcinomas are characterized by the absence of GST-pi protein due to a heavy methylation of the promoter of GSTP1 gene (encoding for GSTpi enzyme), which prevents its transcription. The prostatic cancer cell line LNCaP retains this molecular characteristic and the GST enzymatic activity in these cells is very low; conversely prostatic carcinoma DU-145 cells, which do not present promoter hypermethylation, express GST-pi protein. We used these cell lines to determine the relative sensitivity to the new anticancer agent brostallicin, currently in Phase II clinical evaluation. The drug showed a differential cytotoxicity against these cell lines, being more effective on DU 145 than on LNCaP cells (IC_{50} 38 and >200 ng/ml, respectively).
DNA methylation can be reverted by using demethylating agents, such as the cytidine analogs 5-aza-deoxycytidine (5-aza-dC) and zebularine, or procaine and procanaimide.
The aim of this work was:
- to test the ability of these agents to restore the expression of GSTP1 gene in LNCaP cells;
- to investigate whether re-expression of GST-pi in these cells was associated with an increased activity of brostallicin.

Although 5-aza-dC is a very strong demethylating agent, its use in combination is limited by its very high cytotoxicity; on the contrary, the more stable cytidine analog zebularine is less cytotoxic and combination treatments with brostallicin were performed.
LNCaP cells were treated with zebularine at the concentrations of 50 μM, 75 μM, 100 μM and 125 μM for 96 or 120 hours. GST total activity measurement was performed on cytosolic proteins, by using the substrate 1-chloro-2,4-dinitrobenzene. Results showed a dose-dependent increase of GST activity.
Sequential administration of zebularine and brostallicin resulted in an increased cytotoxicity of brostallicin on LNCaP cells. In vivo experiments are in progress.
The two reported hypomethylating agents, procainamide and procaine did not show, in these cells, strong hypomethylating activity and as a consequence, did not increase the activity of brostallicin.

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The novel DNA cross-linking agent SJG-136 (NSC 694501) exhibits potent, selective and p53-independent cytotoxicity in human chronic lymphocytic leukaemia cells

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SJG-136 (NSC 694501) is a novel chemotherapeutic agent that binds in a sequence-selective manner in the minor groove of DNA. It is structurally distinct from other clinically-used DNA cross-linking agents and has exhibited a unique multi-log differential pattern of activity in the NCI 60 cell line screen (i.e. is COMPARE negative to other cross-linking agents). Given this profile, we undertook a pre-clinical evaluation of SJG-136 in primary tumour cells derived from 34 chronic lymphocytic leukaemia (CLL) patients. SJG-136 induced apoptosis in all the CLL samples tested with mean LD_{50} and LD_{90} values (the concentration of drug required to kill 50% and 90% of the cells) of 9.06 nM and 43.09 nM respectively. SJG-136-induced apoptosis was associated with the activation of caspase-3 and was partially abrogated by the caspase-9 inhibitor Z-LEHD.FMK but not the caspase-8 inhibitor Z-IETD.FMK. Importantly, its cytotoxicity was undiminished in CLL cells derived from previously treated patients, those with unmutated V_H genes and those with p53 mutations (P = 0.17; P = 0.63; P = 0.42 respectively). Furthermore, SJG-136 did not trigger the phosphorylation of p53 or the up-regulation of GADD45 expression in CLL cells whereas the cross-linking agent chlorambucil elicited both of these effects. This indicates that SJG-136 cross-linking adducts are not subject to p53-mediated DNA excision repair mechanisms in CLL cells. Taken together these data demonstrate a novel, p53-independent mechanism of action for SJG-136 that appears to circumvent the effects of poor prognostic markers in CLL. This unique cytotoxicity profile warrants further investigation and supports the use of this agent in phase I clinical trials.

Marine compound

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Kahalalide F (KF) induces apoptosis-independent cell death that involves ErbB3 downregulation and inhibition of Akt signalling

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Kahalalide F (KF) is a novel, marine-derived antitumor agent that is currently undergoing phase II clinical trials, however the mechanism of action is not well understood. We show that KF caused rapid and potent cytotoxic effects in the breast cancer cell lines SKBR3 and BT474. Several markers of caspase-dependent apoptosis were negative after KF exposure, including the externalization of phosphatidyl serine, release of cytochrome C out of mitochondria and the cleavage of caspase-3 and PARP. Moreover, molecular and chemical inhibitors of caspases or cathepsins failed to protect against KF-cytotoxicity. These data indicate that KF-induced cytotoxicity is independent from the basic apoptotic machinery, resembling necrotic cell death. Furthermore, the sensitivity to KF in a panel of human tumor cell lines derived from breast (SKBR3, BT474, MCF7), vulval (A431), non-small cell lung (H460, A549, SW1573, H292), and hepatic (Skhep1, HepG2, Hep3B) carcinoma, significantly correlated with protein expression levels of ErbB3 (HER3) but not other ErbB receptors. Downregulation of ErbB3 expression and inhibition of the PI3K-Akt/PKB signaling pathway was observed in KF-sensitive cell lines within 4 h exposure to KF. Conversely, ectopic expression of a constitutively active mutant of Akt protected against KF cytotoxicity in SKBR3 cells. Moreover, a KF-resistant subline of the colon carcinoma cell line HT29 expressed significantly reduced levels of all ErbB receptors (ErbB1–4) relative to the parental cell line. In conclusion, ErbB3 and Akt are major determinants of the cytotoxic activity of KF *in vitro*. The potential impact of the role of ErbB3/Akt as determinant for clinical response to KF should be considered in upcoming phase II studies.

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The marine, anticancerous compound dehydrothyrsiferol affects integrin mediated adhesion of human breast cancer cells

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We have previously shown that dehydrothyrsiferol (DT), a triterpenoid isolated from a marine, red alga, induces dose and cell type dependent apoptosis in human breast cancer cell lines. Within this process, our group observed detachment of entire cell clusters leading to programed cell death. Integrins are transmembrane, heterodimeric receptor molecules which are frequently involved in cell–extracellular matrix (ECM) interactions through which they mediate a wide range of biological processes, including vascularization, differentiation, and apoptosis. Therefore, our previous findings suggest a potential interference of DT in the bidirectional (inside-out and outside-in) integrin signaling complex. In adhesion assays on collagen and fibronectin coated plastic, DT but not the standard chemotherapeutics doxorubicin and taxol reduced the basal adhesion of estrogen receptor negative MDA MB 231 breast cancer cells via the integrins alpha2beta1 (collagen receptor) and alpha5beta1 (fibronectin receptor) in a dose-dependent manner. Maximum inhibition of adhesion (60%) was reached by incubating the cells with 20 myg/ml of DT for 30 min. A cytotoxic effect was excluded by analyzing propidium iodide uptake of cells treated for even 1 h with the same concentrations as used in the adhesion assays. Flow cytometric analysis revealed that the basal cell surface expression levels of both integrins were not altered by the presence of DT, thereby ruling out protein expression changes as the cause for the reduction of cell adhesion to ECM. To examine changes in the binding capability of the integrin alpha2beta1 in MDA MB 231 cells, we established a flow cytometric assay using a fluorescent, soluble collagen. The treatment with 20 myg/ml DT for 30 min caused a significant reduction of 43±12% (n=7) in the basal collagen binding of this cell type. Taken together, these data suggest that the marine compound DT modulates the signaling through integrins in MDA MB breast cancer cells which may explain, at least in part, the previously observed apoptotic effect, converting