Protein – protein interaction

435 POSTER

Therapeutic potential of a potent and selective antagonist of the MDM2-p53 interaction (MI-219) in combination with traditional chemotherapy

S. Shangary[1], D. McEachern[1], M. Liu[1], R. Miller[1], S. Qiu[1], Z. Nikolovska-Coleska[1], S. Wang[1]. [1]*University of Michigan, Internal Medicine, Ann Arbor, MI, USA*

Background: Reactivation of p53 by blocking the MDM2-p53 interaction is a promising therapeutic strategy for cancers retaining wt p53. We recently reported MI-219 as a potent small molecule inhibitor of the MDM2-p53 interaction which inhibits tumor growth via activation of p53 (Shangary et al. PNAS 2008 105: 3933–3938). Since MI-219 has >10,000-fold weaker affinity for MDMX versus MDM2, MDMX may not allow full reactivation of p53 by MI-219. We investigate here the combination of MI-219 with agents such as chemotherapy drugs which induce MDMX degradation as a rationale approach for enhancing the anti-tumor activity of MI-219. We also compared the effect of MI-219, chemotherapy and ionizing radiation (IR) on accumulation/activation of p53 and apoptosis induction in normal mouse tissues.
Materials and Methods: (1) Prostate (LNCaP) and colon cancer (RKO, HCT-116) and melanoma (SK-Mel-147, SK-Mel-103, SK-Mel-19, MALME-3M) cell lines with wt p53, (2) FP-based MDM2 and MDMX binding assays, (3) cell cycle and apoptosis analyses by flow cytometry, (4) protein–protein interaction by co-immunoprecipitation, (5) Apoptosis, p53 expression and activation in tissues by TUNEL, IHC and immunoblotting, and (6) a mouse xenograft model of SK-Mel-147 human melanoma.
Results: MI-219 induced p53 upregulation and activation in normal and cancer cells with wt p53, and induced partial degradation of MDMX in cancer cells with wt p53. Downregulation of MDMX by RNAi sensitized cancer cells to induction of apoptosis by MI-219. Chemotherapy drugs, such as anthracyclines, etoposide, and platinum-based drugs induced MDMX degradation and markedly enhanced cancer cell apoptosis by MI-219. The effect of MI-219 alone on xenograft tumor growth was minimal, of doxorubicin and cisplatin alone was partial, while dacarabazine alone had no effect. Combination of each drug with MI-219 significantly enhanced the anti-tumor activity of MI-219. Furthermore IR and irinotecan, but not MI-219, induced robust apoptosis in small intestinal crypts and thymus. Although all agents activated p53, IR and irinotecan, but not MI-219, induced robust p53 accumulation.
Conclusion: Combination of MI-219 with chemotherapy drugs which induce MDMX degradation is a promising therapeutic strategy for treating multiple tumor types, even those in which MDMX can be degraded by MI-219. Accumulation and activation of p53 by MI-219 is different from that by genotoxic agents and may contribute to their different toxicities.

436 POSTER

The design, synthesis and antitumour evaluation of novel small molecule inhibitors of the Dishevelled PDZ domain

H. Kadri[1], T.C. Dale[2], K.B.R. Ewan[2], A.D. Westwell[1]. [1]*Welsh School Of Pharmacy, Pharmacy, Cardiff, United Kingdom;* [2]*Cardiff School Of Biosciences, Biosciences, Cardiff, United Kingdom*

The Wnt signalling pathways consist of a complex network of proteins whose aberrant activation has been implicated in tumorgenesis.[1] The PDZ domain of the intracellular Dishevelled (Dvl PDZ) protein binds directly to the transmembrane Frizzled (Fz) protein to relay Wnt signals to downstream components of the beta-catenin degradation complex. We therefore anticipated that an inhibitor of the Dvl PDZ domain would effectively block oncogenic Wnt signalling.
The crystal structure of the dishevelled PDZ domain was used to rationalise the inhibitory activity of a known Dvl-Fz7 interaction inhibitor (FJ9) and closely related anlogues (Fig 1).[2,3] This enabled us to establish the main molecular contacts and key residues mediating this interaction and subsequently a pharmacophore model was generated. Virtual compound library screening combined with structure–activity studies using several scoring functions from commercial and in house modelling programs (MOE, SYBYL) were performed to successfully select promising candidate molecules. Furthermore, protein–protein interaction assays have been developed in house involving the use of bead-based proximity-dependent chemical energy transfer luminescent assays (AlphaScreen). Mechanistic studies will also be used to determine if these compounds alter cell proliferation or apoptosis.
The combination of the robust structure-based virtual screening tools and in house protein–protein interaction assays forms a solid platform for the identification of novel small molecule inhibitors of the dishevelled PDZ domain.

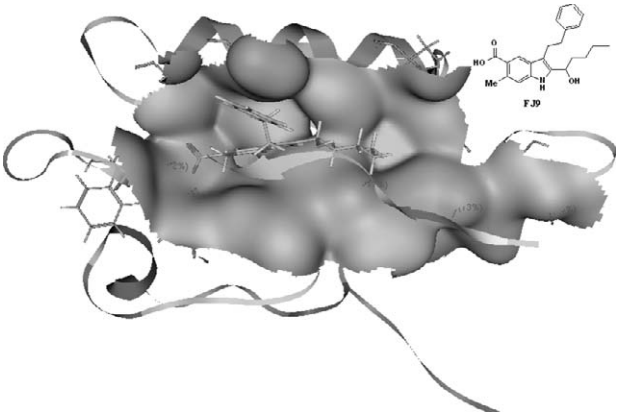

Fig 1: Compound (FJ9) and its interactions with Dvl PDZ domain

References

[1] Reya T, Clevers H. (2005). Nature, 434: 843−50.
[2] Cheyette B.N.R, et al. (2002). Dev. Cell 2, 449–461.
[3] Fujii N, et al. (2007). Cancer Res, 67, 573.

437 POSTER

MDM2 and MDMX status as a determinant of in vitro cellular sensitivity to MDM2 antagonists in human tumour cell lines

X. Lu[1], J. Liu[1], A. Watson[1], I.R. Hardcastle[1], R.J. Griffin[1], B.T. Golding[1], D.R. Newell[1], J. Lunec[1]. [1]*Northern Institute for Cancer Research, Newcastle University, Newcastle uopn Tyne, United Kingdom*

Overexpression of p53 negative regulators MDM2 and MDMX, which in the case of MDM2 is often associated with gene amplification, is frequently found in human tumours, such as osteosarcoma, neuroblastoma and retinoblastoma, and contributes to tumourigenicity by inhibiting p53 tumour suppressor activity. Small molecular weight MDM2 antagonists have recently been shown to disrupt the p53-MDM2 interaction and restore p53 function in tumour cells. To investigate the relationship of MDM2 and MDMX status to *in vitro* cellular sensitivity to MDM2 antagonists (Nutlin, MI-63 and Isoindolinone compound NU8354A), six p53 wild-type human tumour cell lines (*MDM2* amplified: NGP, LS, NB1691 and SJSA-1; and *MDM2* non-amplified: SJNB1 and U2OS) and a pair of isogenic human colorectal cancer cell lines with (HCT116 p53+/+) or without (HCT116 p53−/−) a functional p53 gene, were examined for their cellular response by western blot analysis and growth inhibition (SRB) assay. Moderate to high MDMX protein expression was found in six out of eight cell lines, including two *MDM2* amplified lines (NGP and LS), whereas MDMX was undetectable in the other two *MDM2* amplified lines (SJSA-1 and NB1691). Activation of p53, and p53 downstream transcriptional targets MDM2 and p21 were observed in all seven p53 wt cell lines. Selective sensitivity of HCT116 p53 +/+ over HCT116 p53−/− was observed for all three MDM2 antagonists with 2 to 6 fold difference in GI50. *MDM2* amplified cells (average GI50: 2.6+0.5 μM) were more sensitive to MDM2 antagonists than non-amplified cells (average GI50: 11.8+2.9 μM; $P < 0.0001$). It is of note that in all cell lines, those expressing MDMX (average GI50: 8.8+2.2 μM) are more resistant to MDM2 antagonists than those in which MDMX was undetectable (average GI50: 2.4+0.8 μM; $P < 0.0001$). However, there was no significant difference in relation to MDMX expression for MDM2 amplified cell lines. The *in vitro* cell line data clearly showed that the relative cellular effects of the MDM2 antagonists is predicted from their potency in cell-free ELISA assay. Increase potency of the MDM2 antagonists in cell-free binding assays was associated with increased specificity for growth inhibition of p53 wild type and *MDM2* amplified cell lines.
In conclusion, the results overall indicate that all three MDM2 antagonists were most active for growth inhibition with *MDM2* amplified cell lines and MDMX overexpression may play an important role in MDM2 antagonist-induced resistance.
This work was carried out as part of the European Commission Framework 6 project no. 037834, DePPICT, “Designing Therapeutic Protein–Protein Inhibitors for Brain Cancer Treatments” (http://www.deppict.eu/home.jsp)