325 POSTER

17(allylamino)-17-demethoxygeldanamycin(17-AAG) suppresses tamoxifen-resistant breast cancer cell growth in vitro

T. Madden, D. Barrow, C.M. Dutkowski, R.A. McClelland, J.M.W. Gee, R.I. Nicholson. *Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Cardiff, UK*

Background: Estrogen receptor alpha (ERα) is an important target in the management of breast cancer. However, despite good initial responses, most tumours become refractory to current antihormonal therapies. We have previously demonstrated that acquired resistance to the antiestrogen tamoxifen *in vitro* (TAMR) is associated with increased reliance on cross-talk between ERα and EGFR/HER2 signalling pathways. A number of the proteins comprising these pathways (e.g. ERαHER2, MAPK and AKT) have been reported to be dependent on the molecular chaperone Hsp90 for their conformational maturation which is critical to maintain signalling integrity. The aims of this study were firstly, to establish if their increased dependence on these so-called Hsp90 'client proteins' rendered TAMR cells more sensitive than their tamoxifen responsive counterparts to the Hsp90 inhibitor 17-AAG and secondly, to determine if TAMR cells acquired resistance to this agent *in vitro*.
Materials and Methods: The cellular and molecular effects of 17-AAG were examined using tamoxifen resistant MCF7 (MCF7-TAMR) and T47D (T47D-TAMR) breast cancer cells *in vitro* in comparison with parental MCF-7 and T47-D cell lines. Coulter counting was used to assess cell proliferation, while expression of the above molecular markers of Hsp90 inhibition were evaluated by Western blotting.
Results: In T47D-TAMR and MCF7-TAMR, cellular levels of several key Hsp90 client proteins including ERα, HER2 and AKT were markedly reduced by 17-AAG. These cells were 30-fold and 4-fold more sensitive to this agent respectively versus their hormone responsive counterparts, with >90% inhibition of growth and/or survival. No viable MCF7-TAMR cells remained in culture after 3 months exposure to 17-AAG. However, in T47D-TAMR, growth inhibition was observed for only 1 month, after which surviving cells resumed proliferation. Following a further 5 months of continuous 17-AAG exposure, a stable T47D-TAMR cell line emerged that exhibited 20-fold reduced sensitivity to 17-AAG and growth equivalent to the parental cell line.
Conclusion: These results demonstrate that 17-AAG is more effective in inhibiting the growth and/or survival of tamoxifen resistant breast cancer cells when compared with their antihormone responsive counterparts. Profound inhibitory effects on ERα, HER2 and AKT are consistent with Hsp90 function being central to the multiple overlapping ERα and EGFR/HER2 signal transduction pathways that circumvent the growth inhibitory effects of tamoxifen in these cells. As such, these data clearly advocate the use of this compound on tamoxifen relapse. Crucially, however, our data also suggest that clinical resistance to 17-AAG may develop in some patients. Studies examining the detailed mechanisms of response and resistance to this exciting agent in breast cancer are ongoing.

326 POSTER

Therapeutic targeting of AKT/ILK signaling in AML

M. Konopleva[1], Z. Zeng[1], Y. Tabe[1], Z. Estrov[3], Y. Lu[4], T.S. Daynard[5], G.B. Mills[4], M. Andreeff[1]. *[1]The University of Texas M.D. Anderson Cancer Cente, Blood and Marrow Transplantation, Houston, USA; [3]The University of Texas M.D. Anderson Cancer Center, Bioimmunotherapy, Houston, USA; [4]The University of Texas M.D. Anderson Cancer Center, Molecular Therapeutics, Houston, USA; [5]QLT Inc., Medicinal Chemistry, Vancouver, Canada*

Functional activation of the Phosphatidylinositol 3-Kinase (PI3K)/Akt signaling pathway provides survival signals for leukemic cells and blockade of this pathway may facilitate cell death. The Integrin-Linked Kinase (ILK) phosphorylates Akt and GSK3 kinase through the interaction between malignant cells and their microenvironment. We have previously demonstrated that PI3K/AKT signaling is aberrantly activated in AML cells (*Leukemia 2004, 18(2):267–75*). Since AKT is the main downstream target of PI3K pathway that regulates cell survival and is selectively activated in leukemic but not in normal progenitors, we assessed the effects of a new AKT inhibitor KP372–1 (QLT/Kinetek Pharmaceuticals, Inc., Vancouver, Canada) in myeloid leukemic cells and primary AML samples. KP372–1 inhibited the kinase activity of AKT in vitro. In intact leukemic cells, KP372–1 decreased phosphorylation of AKT on both S473 and T308 and abrogated phosphorylation of downstream p70S6 kinase, while phospho-ERK was not affected. KP372–1 (0.25–1 μM) induced rapid (3–6 hrs) mitochondrial depolarization followed by phosphatidylserine exposure, caspase activation and apoptosis of leukemic cells. Western blot analysis revealed cleavage of caspases-8, -9, and -3. KP372–1 treatment further resulted in de-phosphorylation of Bad and Foxo3A transcription factor, whereas the protein levels of Bcl-2, BAX, Bcl-X_L, MCL-1, XIAP and survivin were not affected. KP372–1 (at 1 μM) induced apoptosis in 17 of 20 primary AML samples at 24 hrs (57±5%), while no apoptosis was induced in normal G-SCF-mobilized peripheral blood cells (n=4). Colony formation of AML blasts (n=5) was reduced to 58±9% at 0.1 μM, 29±10% at 0.5 μM, and 8±3% at 1 μM indicating that KP372–1 induced an irreversible growth arrest or apoptosis in primary AML samples.
We have previously demonstrated that the bone marrow microenvironment plays a crucial role in the pathogenesis of AML by influencing tumor growth, survival, and drug resistance. We now demonstrate that co-culture of leukemic cells with bone marrow stromal cells results in activation of different signaling pathways in leukemic cells including AKT, Stat-3, and MAPK and prevents spontaneous apoptosis. This effect requires direct interaction between stromal and leukemic cells, and separation of leukemic cells from stromal cells by a permeable filter abrogated the supporting effect of stromal cells. Since ILK directly interacts with integrins we examined the effects of the ILK inhibitors on leukemic cells growing in direct contact with bone marrow stromal cells. KP006 is a selective ILK inhibitor that inhibits phosphorylation of AKT on S473 (PDK2/ILK site) but not on Thr 308 (the PDK1 site). Exposure of leukemic NB4 cells alone or even more so of NB4 cells co-cultured with stromal cells to the ILK inhibitor KP006 (10 μM) completely abrogated growth of leukemic cells via massive induction of apoptosis.
Collectively, these data suggest that abrogation of ILK/AKT signaling induces apoptosis, abrogates clonogenic growth of primary AML and may overcome protective effects of the bone marrow microenvironment. This may also ameliorate chemoresistance in leukemias.

327 POSTER

Schedule-dependent inhibition of HIF-1alpha protein accumulation, angiogenesis and tumor growth by topotecan in U251 glioblastoma xenografts

A. Rapisarda[1], B. Uranchimeg[1], M. Hollingshead[2], J. Zalek[2], C.A. Bonomi[2], S.D. Borgel[2], J. Carter[2], R. Shoemaker[2], G. Melillo[1]. *[1]SAIC Inc., National Cancer Institute at Frederick, DTP-Tumor Hypoxia Laboratory, Frederick, USA; [2]National Cancer Institute, DTP, Frederick, USA*

We have previously reported that topotecan, a topoisomerase I poison, inhibits HIF-1 transcriptional activity and HIF-1α protein accumulation in several human tumor cell lines in vitro. Here we investigated the effect of topotecan on HIF-1 dependent responses in xenograft tumors. Nude mice were challenged with U251-HRE cells (stably transfected with a plasmid containing the luciferase reporter gene under control of a HIF-1 inducible promoter) and treated with topotecan using a chronic schedule (1mg/kg QDx10). In vivo bioluminescence was monitored using the Xenogen® technology. Expression of HIF-1-dependent genes, i.e. VEGF, GLUT-3, and PGK-1 mRNA, was analyzed by real-time PCR; microvessel density (MVD) and HIF-1α staining were evaluated by IHC at the end of the experiment. Topotecan decreased HIF-1-dependent luciferase expression relative to untreated controls. In untreated mice the luciferase signal increased steadily with tumor growth. Notably, we observed a sustained inhibition of luciferase expression in the group treated with topotecan (15574 photons/sec/mg tumor relative to 34001 photons/sec/mg tumor in the control group). In addition, VEGF mRNA expression measured on tumor lysates decreased by 97% relative to control mice. Consistent with this data, GLUT-3 and PGK-1 mRNA expression were decreased in the group treated with topotecan, whereas ODC mRNA expression, which is hypoxia-dependent but not HIF-1-inducible gene, remained unchanged. In agreement with these results, we observed a marked reduction of HIF-1α protein accumulation in tumor sections from treated mice compared to the untreated controls. We also examined the expression of murine VEGF mRNA by real-time PCR. Interestingly, topotecan significantly decreased the expression of murine VEGF, relative to untreated mice. In addition, we observed a significant decrease in tumor growth and a decreased vascularity of tumors, as assessed by MVD, in the group treated with topotecan relative to untreated mice.
In conclusion, these results suggest that topotecan, administered in a chronic schedule, inhibits HIF-1α protein accumulation *in vivo*, angiogenesis and tumor growth suggesting a possible clinical application of topotecan to target HIF-1.