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Author(s)	Lam, SK; Leung, LL; Ho, JCM
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Massive degradation in FGFR/Akt/Erk signalling by arsenic trioxide and FGFR inhibitor PD173074 in squamous cell lung carcinoma SK-MES-1

SK Lam, LL Leung, JCM Ho
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Lung cancer is the top cancer killer. Squamous cell carcinoma (SCC) represents the second most common histological subtype of lung cancer. Arsenic trioxide (ATO) has been demonstrated to inhibit tumour growth in lung adenocarcinoma and initiate apoptosis in acute promyelocytic leukaemia. Fibroblast growth factor (FGF) receptor (FGFR) amplification is shown in some SCC. FGFR inhibitor (eg PD173074) has been developed to inhibit FGFR.

Methods: The combination effect of ATO and PD173074 was studied using a cell line with FGFR amplification: SK-MES-1. The effect of ATO and/or PD173074 on cell viability and protein expression was studied by MTT assay and Western blot, respectively. Cell cycle arrest, phosphatidylserine externalisation, and mitochondrial membrane depolarisation were monitored by flow cytometry. FGFR knockdown was performed with siRNA targeting FGFR. Proteasome inhibitor (MG-132) was used to study the degradation mechanism. The in-vivo effect of ATO and/or PD173074 was investigated with a nude mice xenograft model.

Results: Combination of ATO and PD173074 reduced cell viability along with increased sub-G1 population, phosphatidylserine externalisation and mitochondrial membrane depolarisation more significantly than single drug alone. In general, downregulation of FGFR, p-Akt, Akt, p-Src, Src, p-c-Raf, c-Raf, Erk, Bcl-2 and survivin as well as upregulation of p-Erk and cleaved PARP were observed upon ATO and/or PD treatment with or without FGF. MG-132 reversed the degradation of Akt, Src, c-Raf and Erk induced by ATO/PD, but not FGFR, which disclosed proteasome degradation system was involved. Downregulation of FGFR, Akt, Src, c-Raf and Erk as well as cleaved PARP elevation induced by ATO and/or PD were confirmed in vivo.

Conclusion: Massive protein degradation (FGFR, Akt, Src, c-Raf and Erk) was induced by ATO and/or PD173074 treatment mainly mediated by activation of proteasome degradation system in SCC cell line SK-MES-1 in vitro and in vivo.

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Development of a clinically relevant in-vitro drug screening platform for chemo-refractory acute myeloid leukaemia patients

SSY Lam, CY Cher, KL Ng, CH Man, ESK Ho, HCH Chow, AMS Cheung, AYH Leung
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: The cure rate of acute myeloid leukaemia (AML) has remained disappointing despite advances in the use of chemotherapy and bone marrow transplantation. The heterogeneous nature of AML in terms of cytogenetic and genetic abnormalities, clinicopathological characteristics, and response to therapies are believed to attribute to the failure of the present standard induction and consolidation regimen. We proposed to develop and optimise an in-vitro drug screening platform using primary AML samples with results validation based on translation into xenograft mouse models and clinical correlation.

Methods: Mononuclear fraction enriched in blasts was extracted from either peripheral blood or bone marrow aspirates of AML patients by density-gradient centrifugation, and cultured in vitro on 96-well plates in various culture conditions and exposed to a library of drugs, including tyrosine kinase inhibitors and chemotherapeutic drugs currently used in treating malignancies. A resazurin-based cell viability agent was used 3 days later in a high-throughput scale to measure their cytotoxic effects on a variety of samples.

Results: Among the 100 samples currently screened, the drugs exhibited varying effects on the primary AML samples. Among the molecules which produced variable effects across the patient samples, we initially verified the FLT3-ITD mutation correlated with sensitivity towards FLT3 inhibitors, and are looking into possible correlation to in-vivo drug response in immunocompromised mice engrafted with the particular samples.

Conclusion: This platform provides a drug response profiling of the heterogeneous AML blasts which could help design personalised medicine for patients in clinical settings.