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p53 Loses grip on *PIK3CA* expression leading to enhanced cell survival during platinum resistance

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ABSTRACT

Tumour suppressor p53, a master transcriptional regulator determines cell fate through preferential activation/repression of a myriad of genes during stress. Till date, activation and preferential binding of p53 on different promoters was reported to be influenced by the nature, strength and duration of stress which mediates its post translational modifications. Cisplatin, a widely used cytotoxic drug represses *PIK3CA* promoter activity and attenuates PI3K/AKT cell survival pathway through p53 activation in sensitive cells. However, very little is understood about the overall mechanism of p53-*PIK3CA* interaction and influence of p53 on the transcriptional status of *PIK3CA* during cisplatin resistance. Here we showed that cisplatin could dynamically alter p53 occupancy between the p53 binding sequences present in *PIK3CA* promoter in ovarian and breast cancer cells. This altered occupancy is dictated by higher acetylation and hyper-phosphorylation at serine 15, serine 20 and serine 46 residues. Interestingly, cisplatin resistant cells when challenged with cisplatin demonstrated abolished *PIK3CA* promoter attenuation, low level of p53 binding, and loss of p53 serine 46 phosphorylation. A phosphorylation deficient S46A mutant failed to repress *PIK3CA* in p53 deficient cells. Elevated expression of Bcl2, *P27* and *cFLIP* indicated a pro-survival state in these resistant cells. Non-invasive real time imaging using two different luciferase reporters showed that cisplatin could simultaneously induce *PIK3CA* attenuation and p53 activation with growth regression in sensitive tumours but not in the resistant tumours where only low level of p53 activation and sustained growth was observed. This is the first report on phosphorylation of p53 serine 46 as a modulator of p53-*PIK3CA* promoter interaction which influences altered binding of p53 at different consensus sequences in the same promoter in response to chemotherapeutic stress. Absence of such modulation in resistant cellular milieu influences cellular homoeostasis in platinum-resistant cells probably due to altered post translational modification of p53.

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1. Introduction

Despite of promising initial response exhibited by cisplatin treatment, recurrence due to acquired resistance is the major obstacle for successful platinum-based therapy in many cancers (Loizzi et al., 2003). Other than the classical mode of action by DNA intercalation, replication stalling followed by apoptosis induction, cisplatin is known to inhibit AKT activation and thereby cell proliferation. Presence of activated Phosphatidylinositol 3-kinase/AKT (PI3K/AKT) signalling is a common characteristics of many cancer cells resistant to drugs like doxorubicin, paclitaxel, 5-flurouracil, etoposide and camptothecin and might be responsible for treatment failure (Knuefermann et al., 2003).

Amplification or activating mutation in *PIK3CA* gene results in AKT activation which in turn promotes cell survival, proliferation and growth signalling and suppresses apoptosis through phosphorylation of multiple targets like Bcl2, Bad and FKHR (apoptosis-related proteins), CREB, TWIST1 and YB1 (transcription factors), ribosomal protein-S6, β-catenin and the mTOR complex components (PRAS40 and mTOR) (Steelman et al., 2011). *PIK3CA* is transcriptionally activated by Foxo3a (Hui et al., 2008), NF-κB (Yang et al., 2008) and YB1 (Astanehe et al., 2009) proteins. Recent study using temperature sensitive SV40 mutant demonstrated that p53 represses *PIK3CA* transcription through direct binding to its promoter in ovarian surface epithelial cells (Astanehe et al., 2008). Subsequently our lab demonstrated that cisplatin and paclitaxel attenuate *PIK3CA* expression through p53 activation and sequential deletion of p53 response elements (RE) in *PIK3CA* promoter abolish this attenuation in ovarian cancer cells and in tumour xenografts as monitored by optical imaging (Gaikwad et al., 2013). Yet, how this p53-*PIK3CA* association is controlled at molecular level remains elusive.

During stress, p53 plays a decisive role in determining cell fate and drives cellular programming either towards growth arrest followed by survival or towards apoptosis. This choice between life and death is dictated by the ability of p53 to preferentially activate or repress particular subsets of genes. Cell cycle arrest governed by p53 is synchronised with transactivation of *P21*, *GADD45A* and transrepression of *CYCLINB1*, *STMN1* and *SHP-1* (Rinn and Huarte, 2011). In response to severe stress, apoptosis is favoured through simultaneous activation of *PUMA*, *BAX*, *AIP* and repression of *BCL2*, *SGK1*, *IGF1R* genes by p53 (Rinn and Huarte, 2011). Such dualistic action of p53 is regulated by extent of protein stabilization, differential affinity towards specific DNA sequences and various post translational modifications (PTMs) (Beckerman and Prives, 2010). Szak et al. (2001) showed delayed transcriptional induction of *PIG3* gene than *P21* and *MDM2* genes is caused by lower affinity of p53 to binding sequences present on *PIG3* promoter compared to sequences present on *P21* and *MDM2* promoters (Szak et al., 2001). Similarly, p53 exhibits higher binding affinity towards *P21*, *FAS* and *SURVIVIN*, moderate affinity to *CDC25C*, *CYCLIN G*, *MDM-2*, *NOXA* and *PCNA* and weak binding to *MDR1*, *PUMA* and *Kai 1* genes in H_2O_2 treated MCF7 cells (Ray et al., 2012). Selective transcriptional regulation of p53-target genes are also facilitated by various post translational modifications (phosphorylation and acetylation) of different residues of p53 (Dai and Gu, 2010). Cisplatin induced DNA damage initiates phosphorylation of p53 at S15 by ATM, ATR and/or DNA-PK kinases followed by phosphorylation at other serine and threonine residues (S20, S33, S37 and T18, T81) (Appella and Anderson, 2001). These modifications escalate binding of p53 to selective target promoters like *P21* and *MDM2* to initiate transcription (Appella and Anderson, 2001). In addition, HIPK2 kinase phosphorylates p53 at S46 which specifically drives transcriptional induction of apoptosis related genes (Di Stefano et al., 2005). Acetylation of p53 is responsible for destabilization of p53-MDM2 interaction and could also lead to abolishment of transactivation of *P21* (Tang et al., 2008). However, acetylation status of p53 following cisplatin treatment is unknown.

Altered p53 PTMs are often associated with tumorigenesis (Dai and Gu, 2010). However, little is understood about reworking of these PTMs and subsequent transcriptional regulation of p53 in drug resistant scenario. Here we showed that cisplatin dynamically altered occupancy of p53 to its response elements (RE) present in *PIK3CA* promoter in ovarian and breast cancer cells. Such alteration was governed by acetylation and hyper phosphorylation of p53 at S15, S20 and S46 residues. Intriguingly, in cisplatin treated resistant cells, p53 exhibited basal level of promoter binding and complete loss of S46 phosphorylation in conjunction with up-regulated Bcl2, *P27* and *cFLIP* expression. A phosphorylation-mimicking mutant S46D (serine to aspartate) but not a phosphorylation-deficient mutant S46A (serine to alanine) was able to attenuate *PIK3CA* expression in p53 null cell line. Real time imaging using two different luciferase reporters showed cisplatin simultaneously induced *PIK3CA* attenuation and p53 activation in sensitive tumours but not in the resistant tumours where only low level of p53 activation was observed. This is the first report on how cisplatin-resistant cells can actively adapt a pro-survival fate by altering influence of p53 upon *PIK3CA* transcription.

2. Material and methods

2.1. Reagents and plasmids

Chemicals and antibodies used in this study are enlisted in Supplementary Tables 1 and 2. The *PIK3CA* sensor (*PIK3CA* promoter driving firefly-luciferase-2-tandem-dimer tomato, *PIK3CA*-fl2-tdt (Gaikwad et al., 2013)), p53-nanoluc fusion reporter (Promega), CMV-fl2-tdt (Gaikwad et al., 2013), modified pcDNA3.1-CMV-β-galactosidase (Clontech) and SN3-p53 were previously reported. To transiently assess the *PIK3CA* promoter activity, the fl2-tdt bifusion reporter was replaced with hrl-egfp (humanised renilla luciferase-enhanced green fluorescence protein). Single site mutant constructs were generated using site directed mutagenesis (SDM) as described earlier (Gaikwad et al., 2013). Deletion constructs containing single site were created using PCR amplification of required sequence and cloning into pcDNA3.1-hrl-egfp vector backbone. All these constructs were confirmed with sequencing.