

COMMENTARY

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Ubiquitination and adaptive responses to BRAF inhibitors in Melanoma

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ABSTRACT

Response to targeted therapies is limited by the activation or inhibition of feedback loops. Here we report the ubiquitin specific peptidase 28/F-box WD repeat-containing protein 7 (USP28/FBW7) complex functions as a negative regulator of mitogen-activated protein kinase (MAPK) pathway by targeting v-raf murine sarcoma viral oncogene homolog B (BRAF) for degradation, a process which is lost in a large proportion of BRAF mutant melanoma patients, resulting in resistance to BRAF inhibitor therapies.

ARTICLE HISTORY

Received 29 June 2018
Revised 30 June 2018
Accepted 4 July 2018

KEYWORDS

USP28/BRAF/Melanoma/
Vemurafenib resistance

Commentary

The generation of inhibitors selectively targeting v-raf murine sarcoma viral oncogene homolog B (BRAF) V600E mutations has revolutionized the treatment of melanoma patients harbouring this lesion with the majority of patients exhibiting marked and prolonged responses. Unfortunately, however, rarely do tumours completely regress limiting the overall response rates of these compounds. One of the major reasons for this is that targeted therapy with kinase inhibitors induces complex compensatory mechanisms, also known as adaptive responses or feedback loops, permitting a small proportion of the original tumour cell population to survive and eventually to proliferate.

Mitogen-activated protein kinase (MAPK) signalling pathway controls various cellular functions including proliferation, transformation, differentiation and migration. To accurately reflect cues from the extracellular environment, MAPK mediated transcriptional responses are tightly regulated by a number of different compensatory mechanisms. One example of which involves the dual specific protein phosphatase (DUSP). DUSP is transcriptionally induced following MAPK activation whereby it functions through a negative feedback loop to dephosphorylate and inactivate ERK1 and ERK2, resulting in downregulation of the pathway.¹ Interestingly, it has been noted that tumour responses in BRAF V600E melanoma patients to BRAF inhibitors are directly correlated with pERK downregulation.² It is therefore unsurprising that activating mutations or upregulation of components of MAPK pathway have been frequently reported as mechanisms of resistance to BRAF inhibitors in the clinic.³

Adaptive responses regulating deubiquitinating enzyme (DUBs) activity has been shown to drive signalling in a number of oncogenic pathways.⁴ In this regard we reasoned that BRAF inhibitor sensitivity in melanoma may be regulated

by DUBs. Our recent results show that ubiquitin specific peptidase 28 (USP28) expression is enhanced following treatment with vemurafenib, a clinically approved BRAF(V600E) inhibitor, whereby USP28 acts in conjunction with the F-box WD repeat containing protein 7 (FBW7) complex to target BRAF for degradation.⁵ In contrast, loss of USP28 expression led to BRAF stabilisation and downstream activation of canonical MAPK pathway (Figure 1). FBW7 is a component of SKP1/CUL1/F-box (SCF) E3 ligase complex and acts as a substrate recognition subunit binding to phosphorylated degron sites of various oncogenes such as MYC, NOTCH1, JUN, Cyclin E.⁶ The interaction of FBW7 with its substrates induces SCF mediated ubiquitination of bound proteins leading to their proteasomal mediated degradation. However, FBW7 itself is targeted by the SCF complex resulting in its own degradation. USP28 interacts with and deubiquitinates FBW7 leading to the stability of FBW7 and consequently promoting degradation of FBW7 substrates. On the other hand, in circumstances where USP28 levels are high, USP28 can deubiquitinate and stabilize both FBW7 and its bound substrate.⁷ Thus, supporting a role for USP28 as both a tumour suppressor and oncogene with our own study validating a tumour suppressor role of USP28 in melanoma.⁸

Recently, inactivating mutations in FBW7 have been identified in melanoma correlating with poor prognosis.⁹ Importantly, our own analysis indicates that USP28 expression is downregulated in a large proportion of melanoma patients with the majority of these patients harbouring mutations in BRAF (V600E), neurofibromatosis type 1 (NF1), neuroblastoma RAS viral oncogene homolog (NRAS), supporting a role for USP28 loss in melanoma progression. Furthermore, *in vitro* and *in vivo* studies of BRAF mutant melanoma cell lines showed that USP28 depletion enhanced phosphorylated ERK levels leading to

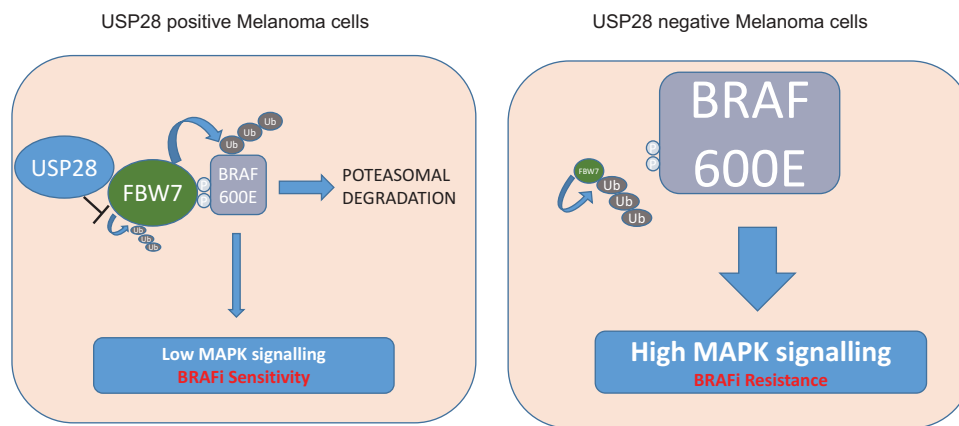


Figure 1. Vemurafenib resistance in melanoma cells lacking USP28. Under normal conditions ubiquitin specific peptidase 28 (USP28) functions to stabilise F-box WD repeat-containing protein 7 (FBW7) resulting in SKP1/CUL1/F-box (SCF) mediated downregulation of targeted substrates including v-ras murine sarcoma viral oncogene homolog B (BRAF, left panel). Under USP28 depleted conditions BRAF stabilisation enhances mitogen-activated protein kinases (MAPK) pathway activation and resistance to BRAF inhibitors (right panel).

decreased vemurafenib sensitivity. Furthermore, diminished USP28 levels inhibited vemurafenib induced apoptosis through the downregulation of pro-apoptotic proteins including BIM. These observations were further supported by the observation of increased time to progression in melanoma patients with high levels of USP28 in response to BRAF/MEK inhibition therapy suggesting a role for USP28 as a potential biomarker for vemurafenib resistance.

In a search to characterize synthetic lethal interactions in melanoma cell lines depleted for USP28 we identified the polo-like kinase 1 (PLK1) inhibitor, rigosertib. Treatment with rigosertib specifically enhanced apoptosis in USP28 depleted cell lines compared to wild type cell lines. Interestingly, rigosertib like drugs such as nocodazole, which are able to induce cell cycle arrest, were projected out in our drug screening analysis as synthetic lethal hits with the loss of USP28 enzyme. Although, recent studies have shown that these anticancer drugs are able to trigger c-Jun N-terminal kinase (JNK) mediated phosphoregulatory circuits to inhibit RAS dependent activation of MAPK signalling,¹⁰ future studies are required to identify the specific mechanism of action of rigosertib in targeting USP28 deleted melanoma cells in a RAS independent activation context in which BRAF(V600E) mutant kinase acts as a monomer to promote the tumorigenic behaviour of cancer cells in melanoma.

At present a number of USP28 inhibitors are under development. As USP28 is differently expressed in different tissues, downregulation of USP28 under certain conditions may be a promising therapeutic strategy to downregulate FBW7 substrates. For example, in intestinal cancers, where USP28 is highly expressed, the FBW7 substrate MYC proto-oncogene (c-MYC) may be a direct target for USP28 deubiquitination activity leading to c-MYC stabilisation in these tissues. Effective targeting of USP28 may lead to c-MYC downregulation and a reversal of c-MYC mediated transformation. However, in tissues such as skin which exhibit low expression of USP28, the enzymatic activity of USP28 is confined to FBW7 leading to the degradation of oncogenes such as BRAF. Deciphering the molecular context of USP28 in different tissues will be of great importance to maximizing the clinical efficacy of USP28 inhibitors. Furthermore, our findings also

point to the use of rigosertib as a potential therapeutic strategy to target USP28 depleted melanoma cells.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Funding

This work was in part supported by the by the National Research Foundation Singapore and the Singapore Ministry of Education under its Research Centres of Excellence initiative.

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