Nature Reviews Urology | Published online 11 Apr 2017; doi:10.1038/nrurol.2017.56

PROSTATE CANCER

Twist and Skp2 castration resistance

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> Skp2 is essential for progression of CRPC and acts by stabilizing Twist and increasing Twist-mediated EMT and CSC acquisition

A study published in *Oncogene* has demonstrated the role of S-phase protein kinase 2 (Skp2) in the progression of castration-resistant prostate cancer (CRPC) via Twist-mediated functions including increased epithelial–mesenchymal transition (EMT) and acquisition of cancer stem cells (CSCs).

The Twist transcription factor is overexpressed in castration-resistant tumour cells and is known to be a key driver towards EMT and CSC acquisition. Furthermore, Twist overexpression leads to resistance to paclitaxel and Twist inactivation inhibits prostate cancer growth and migration. Despite the fact that 90% of malignant prostate cancers exhibit upregulation of Twist, the mechanisms by which this arises is unclear. The F-Box protein Skp2 is a component of the SCF E3 ubiquitin ligase complex that is overexpressed in prostate cancer, and correlates with tumour stage, disease recurrence, and worse patient survival. Evidence from xenograft studies suggests that Skp2 also has a role in castration resistance.

In order to investigate the roles of Skp2 in prostate cancer, Ruan and co-workers began by crossing *Skp2*-null mice with TRAMP mice (which spontaneously develop prostate cancer) and compared tumorigenesis in wild-type, TRAMP, and TRAMP/*Skp2*$^{-/-}$ models. At analysis, 100% of TRAMP mice had developed tumours, compared with just one of 15 age-matched TRAMP/*Skp2*$^{-/-}$ mice, suggesting a role for Skp2 in tumorigenesis. Further follow-up monitoring revealed that *Skp2* knockout inhibited metastasis and markedly improved overall survival, suggesting that targeting Skp2 could be a potential approach to prevent or treat CRPC.

As Twist has been shown to be correlated with CRPC metastases and disease progression, the team went on to further study Skp2–Twist crosstalk using short hairpin RNA depletion of Skp2 expression in two prostate cancer cell lines, PC3 and 22Rv1. Western blot showed significantly reduced Twist expression in Skp2-knockdown cells, but real-time (RT)-PCR revealed that *Twist* mRNA levels remained normal, showing that Skp2 was not involved in Twist transcription, but that the effect was post-transcriptional.

Leading on from this conclusion, and based on the fact that Skp2 is a component of the SCF E3 ligase complex and, therefore, regulates substrates via ubiquitination, the group decided to investigate whether the post-transcriptional effect of Skp2 on Twist was via ubiquitination. An *in vivo* ubiquitination study showed that Skp2 promoted Twist ubiquitination and stabilized Twist protein expression. When this assay was repeated on SCF-E3-ligase-dead mutants, promotion of Twist ubiquitination was impaired, suggesting that Skp2 is a mechanism of Twist activation.

As Twist is a known inducer of EMT, the researchers went on to investigate the transcription levels of EMT markers. They observed that *Skp2* knockdown in PC3 cells resulted in increased levels of the epithelial marker E-cadherin, but reductions in the mesenchymal markers N-cadherin, vimentin, and S100A4. These observations were also reflected in protein levels of EMT markers, with reduced levels of mesenchymal markers N-cadherin, vimentin and Zeb1 in *Skp2* knockdown cells. Furthermore, Skp2 depletion was shown to result in reduced Twist expression in immunohistochemical studies of knockout mouse tissues, emphasizing the important role of Skp2 in Twist regulation and EMT *in vivo*.

To complete their studies, Ruan *et al.* investigated whether Twist overexpression in PC3 and 22Rv1 cells had an effect on CSC generation using a tumour-sphere-forming assay. Twist overexpression resulted in the formation of larger and more numerous tumour spheres, suggesting that cancer stemness correlates with Twist expression. Owing to the previous studies' evidence of Skp2 as an upstream regulator of Twist, they also studied the effect of Skp2 deficiency, showing that it reduced the size and number of tumour spheres. Accordingly, Skp2 silencing also sensitized CRPC cells towards chemotherapy, with induction of responses to doxirubicin in CRPC cells as well as increased sensitivity to paclitaxel in 22Rv1 cells.

"It will be interesting to further investigate whether genetic Skp2 and/or pharmacological Skp2 inactivation serves as an effective way to target CRPC progression," commented corresponding author Dr Hui-Kuan Lin.

These compelling data show for the first time that Skp2 is essential for progression of CRPC and acts by stabilizing Twist and increasing Twist-mediated EMT and CSC acquisition. However, the story might not end there, as the roles of Twist are far reaching: "Twist has versatile roles in cancer development," explains corresponding author Dr Lori Chan. "Besides EMT, Twist regulates tumour progression independently of EMT, including the induction of stem cell regulator Bmi1 and activation of metastasis facilitators Rac1 and Src. It would be interesting to further explore whether genetic or pharmacological inhibition of Skp2 also mitigates these Twist-mediated activities."

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ORIGINAL ARTICLE Ruan, D. *et al.* Skp2 deficiency restricts the progression and stem cell features of castration-resistant prostate cancer by destabilizing Twist. *Oncogene* http://dx.doi.org/10.1038/onc.2017.64 (2017)