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PROSTATE CANCER

On the right path—Stat3 signalling controls the ARF–Mdm2–p53 tumour-suppressor pathway

New data, published in *Nature Communications*, has suggested a previously unknown function for STAT3 in prostate cancer tumorigenicity and metastatic progression.

Penick and colleagues have shown that genetic inactivation of *Stat3* or *IL-6* in a *Pten*-deficient prostate cancer mouse model accelerates cancer progression and leads to metastasis. The investigators observed that mice with conditional loss of *Pten* in the prostate epithelium had markedly increased prostatic Stat3 expression compared with wild-type mice, along with increased phosphorylation of IL-6Rα in tumour cells. To investigate this observation and the role of Stat3 in prostate cancer formation, the team created mice with concomitant prostate-epithelium-specific loss of *Pten* and *Stat3*. Mice with this double deletion showed accelerated prostate cancer formation, with an up to sixfold increase in tumour volume compared with tumours from *Pten*-deficient mice, increased numbers of Ki-67 positive cells and decreased numbers of apoptotic cells. Double-deletion mice developed high-grade, poorly differentiated prostate cancer with liver and lung metastases. By contrast, *Pten*-deficient mice only showed local invasion into seminal vesicles and did not develop metastases.

In vitro, primary cells derived from *Pten*-deficient tumours and treated with short-hairpin RNA to knock down *Stat3* were significantly more invasive in a transwell invasion assay and also in an organotypic, physiologically relevant, 3D cancer model than their control counterparts. These cells also showed increased anchorage-independent cell growth compared with untreated cells.

Experiments to corroborate these observations in the human prostate cancer cell line RWPE-1 showed that combined knock down of *STAT3* and *PTEN* increased the invasiveness of these

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cells. Re-expression of STAT3 in PC3 prostate carcinoma cells, which naturally lack STAT3, resulted in reduced foci formation and decreased cell numbers. These observations are consistent with a tumour-suppresive role for STAT3 in prostate cancer.

Loss of *Stat3* and *Pten* in the mouse model led to a cancer phenotype that is very similar to that of cancer deficient in *p53* and *Pten*. This loss of expression resulted in downregulation of p53 and $p19^{ARF}$ in the prostate epithelium as well as an increase in Mdm2 levels. These findings suggest that loss of Stat3 promotes prostate cancer development by bypassing senescence, which is regulated by the $p19^{ARF}$–p53 axis, and that the tumour suppressive capacity of Stat3 in senescent tumour cells is reliant on the $p19^{ARF}$–Mdm2–p53 tumour-supressor pathway.

Analysis by immunoblot and immunohistochemistry showed a positive correlation between Stat3 and $p19^{ARF}$ and prostate tissue from *Stat3*-deficient mice had significantly decreased $p19^{ARF}$ expression—suggesting that $p19^{ARF}$ could be a novel, directly regulated target of Stat3. *In silico* analysis of the $p19^{ARF}$ promoter predicted two Stat3-binding sites and chromatin immunoprecipitation showed increased Stat3 binding to the $p19^{ARF}$ promoter in *Pten*-deficient primary mouse tumours.

Investigation of the role of IL-6, which is a major regulator of Stat3 that has therapeutic relevance, in Stat3 activation revealed that codeletion of *IL-6* and *Pten* resulted in increased tumour size and grade, similar to that observed *Stat3*–*Pten*-deficient mice.

In patients with prostate cancer, low IL-6 tumour expression correlated with an increased risk of biochemical recurrence and patients with high STAT3 levels had a good prognosis. STAT3 expression correlated with $p14^{ARF}$ expression in 204 patient-derived samples, and combined loss of these two proteins predicted worse outcomes and metastatic progression in these patients. Lower STAT3 levels correlated with increased Gleason score and multivariate analysis showed that $p14^{ARF}$ is a reliable, independent prognostic marker for prostate cancer, with a twofold higher hazard ratio than Gleason score.

Zoran Culig, a member of the research team, told *Nature Reviews Urology,* "These data have numerous implications for the treatment of prostate cancer. Empirical anti-IL-6 or anti-STAT3 treatment without knowledge of STAT3-regulated targets is unlikely to be successful, owing to the tumour-suppressive role of STAT3." He concluded "$p14^{ARF}$ status should be assessed before the initiation of anti-STAT3 treatment, to ensure it is appropriate."

Louise Stone

Original article Pencik, J. *et al*. STAT3 regulated ARF expression supresses prostate cancer metastasis. *Nat. Commun.* doi:10.1038/ncomms8736