as cytokines/chemokines were increased in PD-L1hi and RAS signaling was elevated in H69M cells, and critical components of the innate immunity system, such as TBK1 and IRF3, as well as cytoxins/chemokines were increased in PD-L1hi and CD44hi subpopulations of H69M cells. PD-L1hi H69M cells reverted phenotypically, but not genomically, to H69 cells, suggesting that innate immunity was epigenetically activated. Expression analysis of endogenous retroviral elements (ERV), which are epigenetically silenced, identified IFNγ-inducible antisense 3' UTR ERVs, called SPARCS, in genes enriched for STAT1 motifs in PD-L1hi H69M cells. Further, PD-L1hi H69M cells exhibited increased upregulation of cytosolic double-stranded RNA (dsRNA) sensing pathways, which activate IFN. Chemoresistant mesenchymal H69 (H69AR), but not parental H69, cells exhibited IFNγ-induced PD-L1 expression and increased chemokinesis and permissiveness to PDARCS loci; moreover, EZH2, which was downregulated in H69AR and H69M cells, was shown to repress IFN-stimulated SPARCS expression in parental H69 cells. Exogenous IFNγ simultaneously induced expression of SPARCS-derived dsRNA and increased levels of activated TBK1 and IRF3, subseqently increasing SPARCS expression, TBK1 activation, and effector cytokine production in H69AR cells, suggesting that SPARCS amplifies the IFN-mediated innate immune signalling feedback loop. Expression of a SPARCS gene signature was enriched in other cancer types, such as renal cell carcinoma and glioblastoma, and correlated with that of genes related to epithelial-to-mesenchymal transition. These findings identify a subclass of ERVs that promotes innate immunity in mesenchymal cells and suggest potential approaches to immunotherapy for other cancers.


**Research Watch**

### Kinases

**Major finding:** Phosphorylation of Ser473 and dual phosphorylation of Ser477/Thr479 activate AKT via distinct mechanisms.

**Approach:** Expressed protein ligation generated semisynthetic AKT1 proteins with site-specific phosphorylation.

**Impact:** Differentially modified forms of AKT have distinct functions that may affect inhibitor development.

### Immunology

**Major finding:** IFN-inducible endogenous retroviruses promote mesenchymal tumor cell-mediated immunosuppression.

**Mechanism:** Loss of EZH2-driven repression of SPARCS activates IFN-mediated innate immune pathways.

**Impact:** Therapeutic targeting of the SPARCS positive feedback loop may enhance cancer immunotherapy.

### AKT1 Can Be Activated via Distinct Phosphorylation-Based Mechanisms

Phosphorylation of AKT1 drives downstream signaling and cancer cell proliferation, and AKT1 inhibitors are in clinical development. In the classic model of AKT1 regulation, AKT1 is recruited to the plasma membrane by PI3K where it is phosphorylated on the C-terminus (Ser473) by mTORC2 and on the activation loop (Thr308) by PDK1, leading to enhanced kinase activity. AKT1 can also be phosphorylated on Ser477 and Thr479, and dual phosphorylation of these noncanonical sites has been suggested to mimic Ser473 phosphorylation. However, analysis of AKT1 regulation has been limited by the lack of methods for purification of specific phospho-modified AKT1. To address this limitation, Chu and colleagues used expressed protein ligation to generate 18 specific AKT1 phospho forms. These semisynthetic proteins were used to determine the structure and mechanisms by which AKT phosphorylation controls its activity. Phosphorylation of Ser473 promoted AKT1 activation via an interaction between the C-tail and the pleckstrin homology (PH) kinase domain linker that relieved AKT1 autoinhibition mediated by the PH domain. In contrast, dual phosphorylation of Ser477 and Thr479 produced an alternative active kinase conformation where AKT1 was activated via an interaction of the activation loop that suppresses PH domain-mediated autoinhibition and weakens the affinity for PI3P. Taken together, these findings suggest distinct modes of AKT activation mediated by specific phosphorylation events, and these findings may have implications for the development of AKT inhibitors in cancer. Moreover, the expressed protein ligation approach may facilitate further studies to broaden understanding of the effects of post-translational modifications on AKT1.

AKT1 Can Be Activated via Distinct Phosphorylation-Based Mechanisms

Cancer Discov 2018;8:1060. Published OnlineFirst August 10, 2018.

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