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The PVT1-MYC duet in cancer

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Abbreviations: Brd4, bromodomain 4; CCDC26, coiled-coil domain containing 26; GSDMC, gasdermin-C; lncRNA, long non-coding RNA; MMTV, mouse mammary tumor virus; MYC, myelocytomatosis; PVT1, plasmacytoma variant translocation 1; TCGA, The Cancer Genome Atlas.

Gain of 8q24, harboring the avian myelocytomatosis viral oncogene homolog (*MYC*), is a frequent mutation in cancers. Although *MYC* is the usual suspect in these cancers, the role of other co-gained loci remains mostly unknown. We have recently found that *MYC* partners with the adjacent long non-coding RNA (lncRNA) plasmacytoma variant translocation 1 (*PVT1*), which stabilizes MYC protein and potentiates its activity.

Genomic copy number gain of the human 8q24 region is found in all major cancers.[1] The 8q24 region is a 'gene desert' that contains the v-myc avian myelocytomatosis viral oncogene homolog (*MYC*), an obvious candidate locus. However, gain/amplification of 8q24 frequently includes *MYC* and the adjacent regions, which contain additional genetic elements whose potential role in the induction of cancer is underinvestigated. Adjacent to *MYC* is plasmacytoma variant translocation 1 (*PVT1*), a long non-coding RNA (lncRNA) that was originally identified as a cluster of breakpoints for viral integration and translocation in T- and B-cell lymphomas.[2] The *PVT1* locus is syntenically conserved between the human and mouse. Although *PVT1* is a mutational hotspot and frequently overexpressed in cancers, its role in tumorigenesis is poorly understood.

Despite the existence of several mouse models of *Myc*, lack of an *in vivo* system that links *MYC* with additional elements in the 8q24 region has been an impediment to understanding the contribution of additional genetic elements in this region to the pathophysiology of cancer. We sought to functionally determine whether gain of *MYC* alone is sufficient to drive tumor formation, or whether other elements in this gene-desert region also play a role in malignancy.[3] We used chromosome engineering of mouse ES cells[4] to develop mice with a single-copy gain of the following regions: (1) an ~1.9 Mb genomic interval that is syntenic to Hu 8q24.21 encompassing *Myc*, *Pvt1*, coiled-coil domain containing 26 (*Ccdc26*), and gasdermin-C (*Gsdmc*); (2) *Myc* only, and (3) *Pvt1*, *Ccdc26*, and *Gsdmc*.

Our first surprise came when mammary glands of mice with gain of Myc/Pvt1/Ccdc26/Gsdmc, but not those with gain of Myc alone, exhibited signatures of increased oncogenic activity and formed mammary tumors with reduced latency in the presence of a Neu transgene expressed under the mouse mammary tumor virus (MMTV) promoter. Also, *Pvt1* mRNA and Myc protein levels were significantly elevated in gain (Myc/Pvt1/Ccdc26/Gsdmc) mammary glands. Knocking down *Myc* or *Pvt1* using siRNAs reduced the proliferation of (Myc/Pvt1/Ccdc26/Gsdmc),MMTVNeu/+ mammary tumor cells, suggesting that lncRNA Pvt1 cooperates with Myc in these tumors. Surprisingly, knockdown of both *Myc* and *Pvt1* reduced proliferation to the same extent as when Myc or Pvt1 were depleted independently in (Myc/Pvt1/Ccdc26/Gsdmc),MMTVNeu/+ cells and human breast cancer cell lines with 8q24 amplification (SK-BR-3 and MD-MBA-231). This led us to hypothesize that *MYC* and *PVT1* may share the same oncogenic pathway in these cells.

SiRNA mediated knockdown of *PVT1* in SK-BR-3 and MDA-MB-231 resulted in significant reduction of MYC protein, but, interestingly, not transcript levels. This suggested that *PVT1* might regulate MYC protein stability. A chase experiment using the protein synthesis inhibitor cycloheximide in cells with and without *PVT1* confirmed that *PVT1* confers increased stability on MYC protein. Further analysis revealed that *PVT1* decreases phosphorylation of MYC at the Threonine 58 residue (MYC^{T58}), a post-translational modification that licenses MYC degradation.[5] We also found that *PVT1* and MYC preferentially co-localize in the nucleus, and co-immunoprecipitation using antibody against MYC can enrich for the *PVT1* transcript, suggesting that *PVT1* and MYC might be a part of a ribonucleo–protein complex in which *PVT1*

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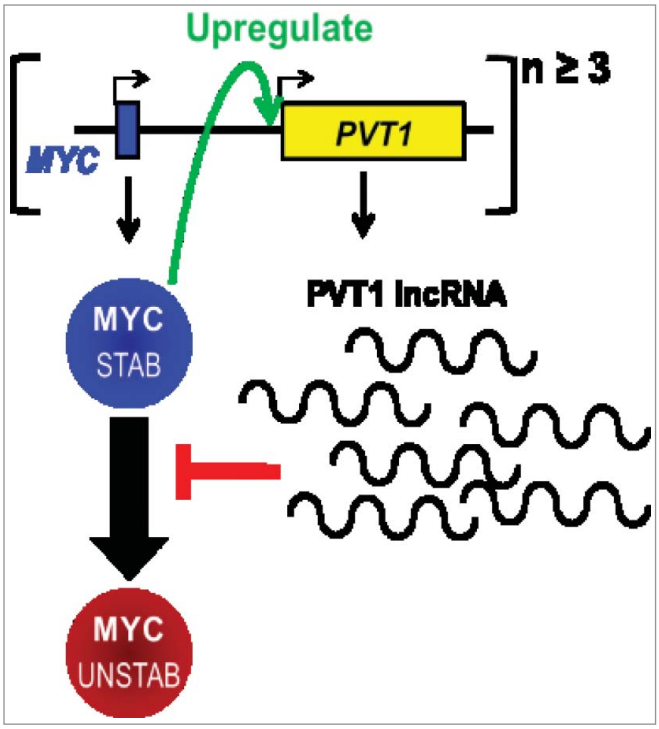

Figure 1. The PVT1-MYC positive feedback axis in human cancer. In cancers with supernumerary copies of 8q24 ($N \geq 3$), the long non-coding RNA (lncRNA) *PVT1* can augment MYC protein stability by attenuating its degradation. The stable MYC protein (MYC STAB) acts as a transcription factor by binding to the canonical E-boxes at the promoter region of *PVT1* and can upregulate *PVT1* RNA expression, thus forming an oncogenic circuit contributing to cancer. MYC UNSTAB denotes degraded MYC.

attenuates phosphorylation at MYC^{T58}, thus increasing its stability.

We investigated whether PVT1/MYC co-operation is a fundamental feature in all 8q24 gain cancers by analyzing genomic data from the cancer genome atlas (TCGA) and the Progenetix database. We identified the subset of cancers with 8q24 gain/amplification, and compared how many of those amplicons contained only *MYC* or *PVT1* or both *MYC* and *PVT1*. We hypothesized that if *PVT1* and *MYC* co-operate in human cancers with 8q24 gain, the majority of these amplicons should contain both *MYC* and *PVT1*. Indeed, 98% of the 8q24 amplicons contained both *MYC* and *PVT1*, confirming that both loci are preferentially amplified/gained concurrently in these cancers. The gold standard of proof for such a co-operation should be co-expression of *PVT1* RNA and MYC protein in primary human cancers. A tissue microarray analysis of 8 primary tumors (lung, colon, rectum, stomach, esophagus, liver, kidney, and breast) revealed a high correlation between *PVT1* RNA and MYC protein expression in these primary tumors. These data provided strong evidence for PVT1/MYC co-operation in different human cancers.

Finally, we examined whether MYC-driven cancer cells are dependent on *PVT1*. The driver mutation in the colorectal cancer cell line HCT116 is a mutant β-catenin gene. A stable β-catenin protein recruits TCF4 to upregulate *MYC* transcription in these cells. Using the CRISPR/Cas9 system,[6] we deleted *PVT1* in these cells. *PVT1*-deficient HCT116 cells are impaired in their tumorigenic potential compared to their wild-type controls. Importantly, we noticed ~ 50% reduction in MYC protein levels in these *PVT1*-deficient cells. Thus, multiple lines of evidence suggest that *PVT1* plays a crucial role in augmenting MYC protein in 8q24 gain cancers. Similarly, a recent study implicates another frequently amplified oncogenic lncRNA called *FAL1* at 1q21 in the stabilization of BMI1 in ovarian cancers, suggesting a broader role of lncRNAs in the fine tuning of oncoproteins in cancer.[7]

We have identified a novel regulation of MYC via the lncRNA *PVT1*, at least in cancers where these loci are co-amplified (**Fig. 1**). Whether *PVT1* plays a role in MYC regulation in 8q24 non-supernumerary cancers remains to be investigated. Additionally, a detailed mechanistic understanding of how *PVT1* RNA regulates MYC protein in cancer cells can be exploited therapeutically. It has been shown that MYC ablation causes regression of K-Ras–mediated lung cancer in mice,[8] suggesting the central importance of MYC in cancers. However, so far it has not been possible to target MYC directly.[9] JQ1, a small molecule inhibitor of bromodomain 4 (Brd4) has recently been shown to indirectly inhibit *MYC* transcription in hematological malignancies.[10] Inhibition of PVT1, thereby fine tuning MYC stability in cancers to precancerous levels with less toxic side effects, could be therapeutically exploited for patients with gain of 8q24.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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