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PCAF, ISX, and BRD4: a maleficent alliance serving lung cancer malignancy

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Tumor progression and malignancy are frequently associated with aberrant activation of epithelial–mesenchymal transition (EMT), which orchestrates dramatic changes in gene expression, involving genetic and epigenetic regulation. External stimuli generated by tumor–stroma interactions need to be adequately processed to specifically alter expression of key EMT transcription factors and associated genes. In this issue of EMBO Reports, Wang and colleagues demonstrate how epigenetic modifiers are utilized to induce EMT and metastasis [1]. Acetylation of intestine-specific homeobox (ISX) by p300/CBP-associated factor (PCAF) induces a cascade that results in *Snail* and *Twist* activation through histone modifications by a novel complex of PCAF, ISX, and bromodomain-containing protein 4 (BRD4). These findings open novel possibilities of therapeutic intervention to inhibit EMT and metastasis in lung cancer.

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See also: **L-T Wang *et al*** (February 2020)

The process of epithelial–mesenchymal transition (EMT) is activated by several key events during development and is hijacked by cancer cells during malignant transformation [2]. A widespread and global change in gene expression is required to accomplish partial or full EMT to form new tissues in the embryo, or to enable tumor cell migration, invasion, metastasis, drug resistance, and the acquisition of stem cell properties, resulting in a fatal outcome. These changes in gene expression are not only mediated by direct activation and repression of individual genes, but also involve epigenetic regulation to sustain these changes [3]. Several external triggers including TGFβ, Ras/MAPK, TNFα, EGF, FGF, HGF, and hypoxia signaling pathways, as well as the core EMT transcription factors (TFs) SNAIL, SLUG, TWIST, ZEB1, and ZEB2, are known to orchestrate the EMT program [2,4]. However, it is still enigmatic how external stimuli are processed intracellularly, which gene regulatory networks are involved, which are the key targets of EMT-TFs, and how epigenetic reprogramming is achieved. Epigenetic gene regulation mainly requires the activity of histone-modifying enzymes. They either mediate tight binding of histones to DNA and locus inactivation, or remodeling of chromatin to loosen histone–DNA interactions to allow histone displacement by TFs and recruitment of the core transcription machinery [5]. Histone acetylation is a key process for epigenetic gene activation employing factors with histone acetyltransferase (HAT) activity, and bromodomain-containing reader proteins that serve as scaffolds for the assembly of multiprotein complexes for gene activation. To execute a specific program like EMT, epigenetic regulators need to be directed to specific chromatin regions of key downstream targets.

In cancer, epigenetic gene control is frequently deregulated. p300/CBP-associated factor (PCAF) is a member of the GCN5-related N-acetyltransferase family of protein acetyltransferases with HAT activity and shows ambiguous or controversial function in tumorigenesis. Bromodomain-containing protein 4 (BRD4) acts as a chromatin reader and mediates binding to acetylated histones. It assists in the formation of multiprotein complexes to link superenhancers and promoters [6]. The aberrant expression of many cancer-associated genes seems to be dependent on BRD4 function. Intestine-specific homeobox (ISX) was previously shown to promote proliferation, tumorigenesis, and immune tolerance in hepatocellular carcinoma (HCC) [7].

Now, Wang and colleagues very elegantly show how the epigenetic regulators PCAF and BRD4 cooperate and are recruited to the promoters of *Snail* and *Twist* for gene activation and to execute the EMT program in lung cancer [1]. In A549 cells, unmodified ISX is located to the cytoplasm with low affinity to PCAF and BRD4. PCAF induces acetylation of ISX at lysine 69 and of BRD4 at lysine 332. Acetylation of both proteins substantially increases the affinity of ISX to BRD4, involving the homeodomain (HD) of ISX and bromodomain 2 (BD2) of BRD4. A ternary complex is formed by binding of PCAF to both acetylated proteins, which subsequently translocate to the nucleus. At the promoters of *Snail* and *Twist*, the HAT activity of PCAF then promotes histone H3 acetylation at lysines 9, 14, and 18. These histone modifications induce chromatin remodeling, which allows the association of BRD4–PCAF with acetylated histone H3K27, ultimately leading to the recruitment of the core transcription machinery, including RNA polymerase 2 (Pol II) and p300/CBP [1]. As a result, *Snail* and *Twist*, as well as EMT-associated genes, such as *Vimentin*, *Fibronectin*, and *Cdh2* (N-cadherin), are upregulated, whereas epithelial genes such as *Cdh1* (E-cadherin) are downregulated (Fig 1A). Very strikingly, components of the PCAF-ISX-BRD4 axis show correlated expression in clinical data sets of NSCLC patients, and elevated expression of PCAF, ISX, or BRD4 is correlated with metastasis formation and poor survival. The specific interaction of PCAF with acetylated ISX and BRD4 might serve as a mode of regulation to guide epigenetic modifiers with many potential target sites to a specific gene

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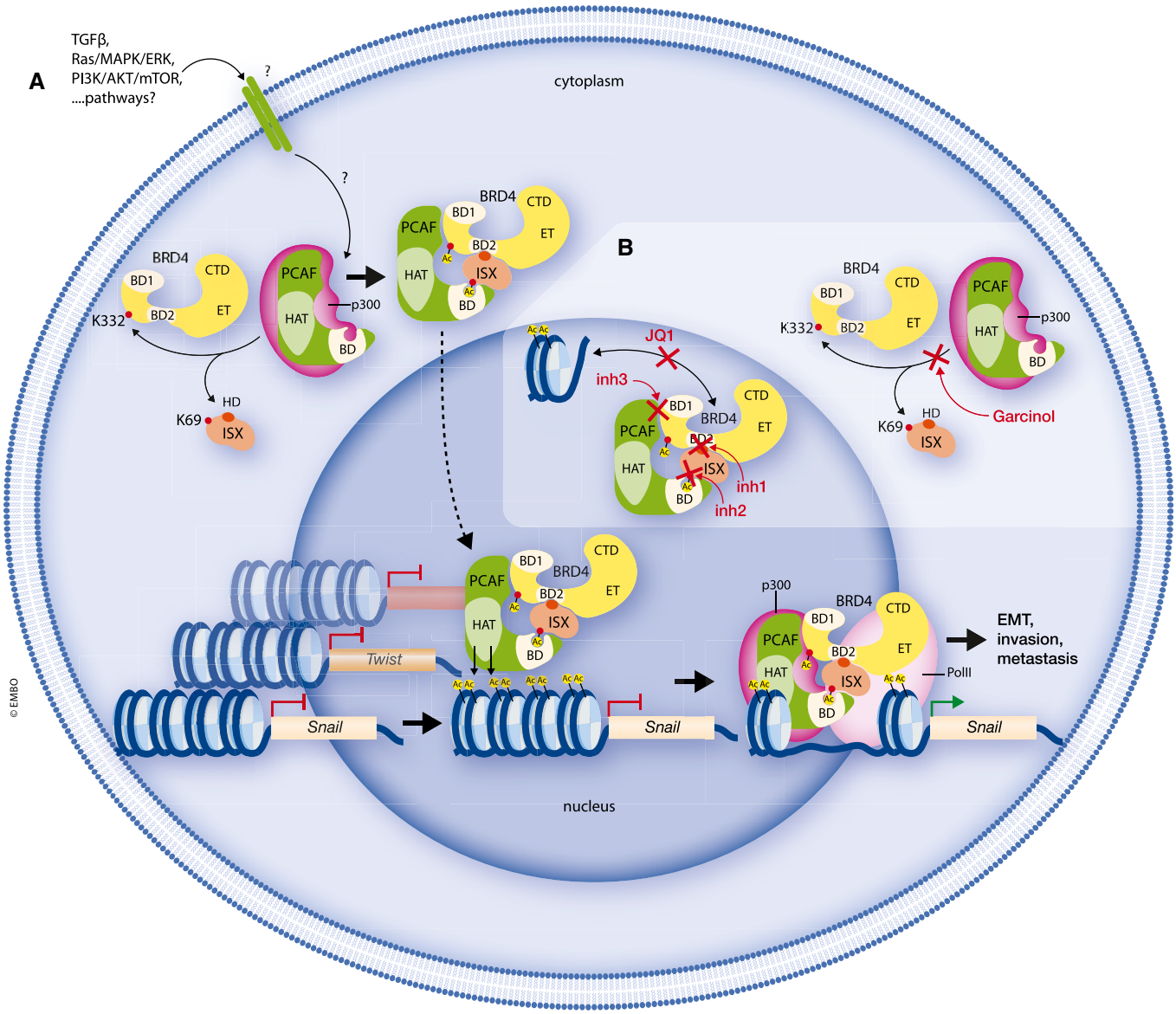

Figure 1. The PCAF-ISX-BRD4 axis induces EMT by driving *Snail* and *Twist* expression in lung cancer.

(A) Model of regulation of key EMT genes by ISX, BRD4, and PCAF. PCAF promotes acetylation (Ac) of ISX and BRD4 in the cytoplasm, which leads to the formation of a ternary complex that translocates to the nucleus. Via histone H3 acetylation on specific genes such as *Snail* and *Twist*, this complex modifies chromatin, and allows histone displacement and recruitment of the transcription machinery, including p300 and RNA polymerase II (Pol II), to activate gene expression for EMT induction. (B) Potential sites of therapeutic intervention to interfere with PCAF-ISX-BRD4 function. The small-molecule inhibitor garcinol prevents acetylation by PCAF; JQ1 disrupts BRD4 binding to acetylated H3 in a global manner. Generation of inhibitors or small peptides (inh1, inh2, and inh3) that block binding of ISX-BRD4, ISX-PCAF, and PCAF-BRD4, respectively, might specifically inhibit EMT and metastasis.

subset that is required for executing distinct programs, in this case the induction of EMT. The study by Wang *et al* now provides a molecular mechanism how this specificity is achieved.

The identification of this novel mode of epigenetic regulation mediated by a PCAF-ISX-BRD4 complex to drive *Snail* and *Twist* expression raises several additional questions. Very strikingly, in A549 cells, the formation of this unique epigenetic regulator complex is induced by ISX overexpression, but not by its non-acetylated counterpart. Moreover, the expression levels of the binding partners of the ternary complex correlate with survival. Hence, it is possible that simply raising ISX expression above a crucial threshold level is sufficient to push PCAF-mediated acetylation and to initiate the downstream cascade toward *Snail*/*Twist* activation. On the other hand, it is likely that external signals such as IL6, that was shown to induce ISX expression in HCC [7], or known pathways with a role in EMT or tumor progression, e.g., TGFβ, PI3K/AKT, or Ras/MAPK signaling, act upstream of ISX and are crucial for facilitating EMT via the PCAF-ISX-BRD4 axis. How this ternary complex is recruited to specific EMT-associated genes is unknown. It is possible that

specific yet unidentified DNA motifs or unique histone modifications guide this complex to its target sites of *Snail* and *Twist* promoters. Interaction of BRD4 with hyper-acetylated chromatin also induces recruitment of the mediator complex via BRD4, serving as a scaffold to promote the assembly of a multi-transcription factor complex at the promoter and at superenhancers [6]. Such BRD4-mediator complexes that bind to *Snail*- and *Twist*-specific superenhancers might be crucial to facilitate recruitment of PCAF-ISX-BRD4 to selected target genes and subsequent activation of EMT. Interestingly, for initiation of the cascade PCAF and presumably also p300 functions are essential for the acetylation of ISX and BRD4, but the binding affinity of PCAF to the non-acetylated versions is very poor. Maybe an additional adaptor protein is required, or PCAF is activating other acetylating proteins that directly act on ISX and BRD4. Alternatively, initial weak binding is stabilized by the acetylation process itself. Further investigation is also required to understand whether this mechanism is restricted to NSCLC or whether it is also active in other tumor entities during malignant transformation.

The current study bears the promise of novel therapeutic interventions to restrict or prevent metastasis formation in tumor patients that show increased expression of PCAF, ISX, and BRD4. Small-molecule inhibitors such as garcinol and JQ1 that block HAT activity of PCAF and binding of BRD4 to acetylated histones, respectively, have already been shown to support standard therapy in preclinical trials [8,9]. However, their mode of action might be too unspecific for efficient therapy, as they also interfere with PCAF and BRD4 functions outside of the PCAF-ISX-BRD4 complex. Accordingly, side effects might prevent treatment with higher doses to efficiently block the formation of this particular ternary complex. In contrast, identification of small-molecule inhibitors or peptides that specifically block the interaction at one or several binding interfaces, e.g., at ISX-BRD4, PCAF-ISX, and PCAF-BRD4 sites, and/or prevent ISX and BRD4 acetylation, will probably act in a more specific manner, without interfering with normal PCAF function at other genes (see Fig 1B). The authors have already demonstrated that mutant ISX, which is no longer acetylated, decelerates tumor growth and metastatic spreading in nude mice upon orthotopic transplantation. Since EMT-TFs such as SNAIL, TWIST, and ZEB1/2 are very difficult to target, such drugs would be beneficial to reduce metastatic load, and to avoid tumor cell spreading in early-stage patients.

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