JCI The Journal of Clinical Investigation

Transcriptional regulation of epithelial-mesenchymal transition

Yingqi Teng, … , Michael Zeisberg, Raghu Kalluri

J Clin Invest. 2007;117(2):304-306. https://doi.org/10.1172/JCI31200.

Commentary

It has become increasingly obvious that the notion of a terminally differentiated cell is likely a simplified concept. Epithelial-mesenchymal transition (EMT), during which epithelial cells assume a mesenchymal phenotype, is a key event occurring during normal development and pathological processes. Multiple extracellular stimuli and transcriptional regulators can trigger EMT, but how such distinct signaling pathways orchestrate the complex cellular events that facilitate EMT is not well understood. In this issue of the *JCI*, Venkov et al. report on their examination of fibroblasts resulting from EMT and describe a novel protein-DNA complex that is essential for transcription of *fibroblast-specific protein 1* (*FSP1*) and sufficient to induce early EMT events (see the related article beginning on page 482). Collectively, their results suggest that this complex is an important regulator of the EMT transcriptome.

of other host proteins that determine the fate of HIV-1 DNA once it has entered the target cell nucleus.

Acknowledgments

Work in the author's laboratory is supported by the NIH, amFAR, and the Elizabeth Glaser Pediatric AIDS Foundation.

Address correspondence to: Paul D. Bieniasz, Aaron Diamond AIDS Research Center, 455 First Avenue, New York, New York 10016, USA. Phone: (212) 448-5070; Fax: (212) 725-1126; E-mail: pbienias@adarc.org.

1. Bieniasz, P.D. 2004. Intrinsic immunity: a front-line defense against viral attack. *Nat. Immunol.* **5**:1109–1115.
2. Goff, S.P. 2004. Genetic control of retrovirus susceptibility in mammalian cells. *Annu. Rev. Genet.* **38**:61–85.
3. Stremlau, M., et al. 2004. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature.* **427**:848–853.
4. Sheehy, A.M., Gaddis, N.C., Choi, J.D., and Malim, M.H. 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature.* **418**:646–650.
5. Naghavi, M.H., et al. 2006. Moesin regulates stable microtubule formation and limits retroviral infection in cultured cells. *EMBO J.* **26**:41–52.
6. Naghavi, M.H., Hatziioannou, T., Gao, G., and Goff, S.P. 2005. Overexpression of fasciculation and elongation protein zeta-1 (FEZ1) induces a post-entry block to retroviruses in cultured cells. *Genes Dev.* **19**:1105–1115.
7. Ganesh, L., et al. 2003. The gene product Murr1 restricts HIV-1 replication in resting CD4+ lymphocytes. *Nature.* **426**:853–857.
8. Boulanger, M.C., et al. 2005. Methylation of Tat by PRMT6 regulates human immunodeficiency virus type 1 gene expression. *J. Virol.* **79**:124–131.
9. Turelli, P., et al. 2001. Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication. *Mol. Cell.* **7**:1245–1254.
10. Zhang, J., Scadden, D.T., and Crumpacker, C.S. 2007. Primitive hematopoietic cells resist HIV-1 infection via $p21^{Waf1/Cip1/Sdi1}$. *J. Clin. Invest.* **117**:473–481. doi:10.1172/JCI28971.
11. Zhang, J., Attar, E., Cohen, K., Crumpacker, C., and Scadden, D. 2005. Silencing p21(Waf1/Cip1/Sdi1) expression increases gene transduction efficiency in primitive human hematopoietic cells. *Gene Ther.* **12**:1444–1452.
12. von Laer, D., et al. 1990. CD34+ hematopoietic progenitor cells are not a major reservoir of the human immunodeficiency virus. *Blood.* **76**:1281–1286.
13. Weichold, F.F., et al. 1998. Neither human immunodeficiency virus-1 (HIV-1) nor HIV-2 infects most-primitive human hematopoietic stem cells as assessed in long-term bone marrow cultures. *Blood.* **91**:907–915.
14. Shen, H., et al. 1999. Intrinsic human immunodeficiency virus type 1 resistance of hematopoietic stem cells despite coreceptor expression. *J. Virol.* **73**:728–737.
15. Xiong, Y., et al. 1993. p21 is a universal inhibitor of cyclin kinases. *Nature.* **366**:701–704.
16. Cheng, T., et al. 2000. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. *Science.* **287**:1804–1808.
17. Zack, J.A., et al. 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell.* **61**:213–222.
18. Waga, S., Hannon, G.J., Beach, D., and Stillman, B. 1994. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature.* **369**:574–578.
19. Lloyd, A.G., et al. 2006. Effect of DNA repair protein Rad18 on viral infection. *PLoS Pathog.* **2**:e40.
20. Llano, M., et al. 2006. An essential role for LEDGF/p75 in HIV integration. *Science.* **314**:461–464.

Transcriptional regulation of epithelial-mesenchymal transition

Yingqi Teng,[1] Michael Zeisberg,[1] and Raghu Kalluri[1,2,3]

[1]Division of Matrix Biology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA.
[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, USA.
[3]Harvard-MIT Division of Health Sciences and Technology, Boston, Massachusetts, USA.

It has become increasingly obvious that the notion of a terminally differentiated cell is likely a simplified concept. Epithelial-mesenchymal transition (EMT), during which epithelial cells assume a mesenchymal phenotype, is a key event occurring during normal development and pathological processes. Multiple extracellular stimuli and transcriptional regulators can trigger EMT, but how such distinct signaling pathways orchestrate the complex cellular events that facilitate EMT is not well understood. In this issue of the *JCI*, Venkov et al. report on their examination of fibroblasts resulting from EMT and describe a novel protein-DNA complex that is essential for transcription of *fibroblast-specific protein 1* (*FSP1*) and sufficient to induce early EMT events (see the related article beginning on page 482). Collectively, their results suggest that this complex is an important regulator of the EMT transcriptome.

During development and adult organ pathogenesis, cells are in a constant state of phenotypic transition. In pathological settings, differentiated adult cells from the kidney, lung, liver, or heart can undergo drastic phenotypic transitions. Such acts are likely undertaken to avoid cell death in a hostile environment. But if an insult, such as organ fibrosis, persists, then such transitions likely become semipermanent.

During epithelial-mesenchymal transition (EMT), epithelial cells gradually lose their epithelial signatures while acquiring the characteristics of mesenchymal cells. EMT is regarded as a critical regulator of metazoan embryogenesis and physiological processes such as wound healing. EMT also contributes significantly in pathologies such as tissue fibrosis and cancer metastasis. Hallmarks of EMT include: (a) the downregulation of cell adhesion molecules such as E-cadherin; (b) the increased expression of MMPs to assist in the degradation of the basement membrane; (c) the activation of the Rac/Rho/Cdc42 family small GTPase to bring about cytoskeleton rearrangement; and (d) the nuclear translocation of several transcription factors including β-catenin and the T cell factor/lymphocyte enhancer factor 1 (TCF/LEF1) complex, Snail1, Snail2, and Twist (1, 2). The adoption of a fibroblast-like transcription profile is crucial for the survival of the cells undergoing EMT. Several key transcription factors have been described (1); however, it is now clear that more such transcriptional regulators are required to govern the complex EMT transcriptome.

Nonstandard abbreviations used: CBF-A, CArG box–binding factor–A; EMT, epithelial-mesenchymal transition; FSP1, fibroblast-specific protein 1; FTS-1, fibroblast transcription site–1; KAP-1, KRAB-associated protein 1.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **117**:304–306 (2007). doi:10.1172/JCI31200.

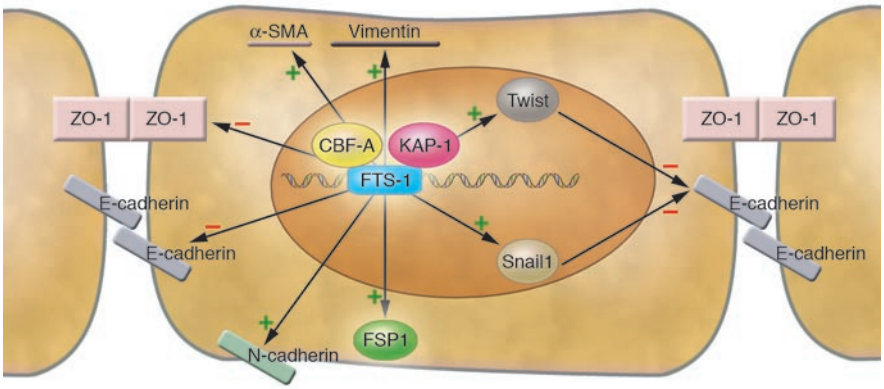

Figure 1
The CBF-A/KAP-1/FTS-1 complex is a master regulator of EMT. In an epithelial cell undergoing EMT, the CBF-A and KAP-1 proteins recognize and bind to the FTS-1 sites in the genomic DNA. The CBF-A/KAP-1/FTS-1 complex controls the expression of a wide spectrum of EMT-responsive genes, possibly via the FTS-1 sites also present in their promoters. Arrows with + or – symbols indicate whether the abundance of a given protein is increased or decreased, respectively, via the action of the CBF-A/KAP-1/FTS-1 complex. ZO-1, zona occludens 1.

A novel protein-DNA complex directly activates fibroblast-specific protein 1 during EMT

The calcium-binding fibroblast-specific protein 1 (FSP1; also known as S100A4) is a crucial facilitator of EMT. The expression of FSP1 marks an early stage of EMT, and blockade of FSP1 expression suppresses the EMT induced by TGF-β and EGF signals (3, 4).

In this regard, a proximal *cis*-acting regulatory element in the FSP1 promoter, capable of interacting specifically with nuclear extracts from the fibroblasts but not the epithelium, was previously identified using EMSA. The element was termed fibroblast transcription site–1 (FTS-1), and the FTS-1 site has been shown to be crucial for the expression of FSP1 in fibroblasts (5).

Now, in this issue of the *JCI*, Venkov et al. report the use of FTS-1 as a probe to identify protein components of the FTS-1 complex in fibroblast nuclear extracts (6). Using 2 independent approaches, EMSA and DNA affinity chromatography, the authors identified 2 proteins, CArG box–binding factor–A (CBF-A) and KRAB-associated protein 1 (KAP-1), present in a complex with FTS-1. Furthermore, the authors report that in a kidney epithelial cell line engineered to conditionally express CBF-A, the expression of CBF-A coincided with formation of the CBF-A/KAP-1/FTS-1 complex and the de novo transcription of the *FSP1* gene.

CBF-A has been described as both a transcriptional activator and a repressor in distinct genomic loci (7–9). Unlike traditional transcription factors, CBF-A recognizes a variety of DNA motifs and has an affinity for both single-stranded and double-stranded DNA (6–8). KAP-1 is generally considered a transcriptional repressor and also interacts with the nucleosome remodeling and deacetylase (NuRD) complex (10, 11). Such evidence suggests that the CBF-A/KAP-1/FTS-1 complex, whose function is likely to facilitate chromatin remodeling, is a direct and major activator of *FSP1* transcription during EMT.

CBF-A as a regulator of the EMT transcriptome and a potential target for drug development

Venkov et al. (6) also show that the effects of CBF-A go beyond just the activation of the *FSP1* gene. Cultured kidney epithelial cells initiate the EMT process when triggered to express CBF-A. These cells assume the spindle-shaped fibroblast morphology, exhibit increased migratory capacity, and display the transcriptional responses associated with EMT (Figure 1). CBF-A thus decreases the abundance of the epithelial cell markers zona occludens 1 (ZO-1) and E-cadherin and increases the expression of fibroblast markers N-cadherin, α-SMA, vimentin, Snail1, and Twist. FTS-1 sites are also present in the promoter regions of multiple genes involved in the EMT process, and these sites are more abundant than TCF/LEF1 sites and E-boxes (Snail family protein–binding sites) (6).

It has been well established that EMT plays a significant role in the pathology of tissue fibrosis and metastasis (1, 2). Bone morphogenic protein 7 has been shown to reverse TGF-β1–induced EMT and ameliorate TGF-β1–triggered fibrosis (12). Nevertheless, potential targets for the future development of drugs that can inhibit EMT need to be further explored.

In this regard, the CBF-A/KAP-1/FTS-1 complex qualifies as a promising therapeutic target upstream of the EMT transcriptome. This notion is supported by the broad presence of FTS-1 sites in EMT-responsive genes and also by the observation that CBF-A controls the expression of important transcription regulators of EMT such as Snail1 and Twist. Therefore, disruption of the CBF-A/KAP-1/FTS-1 complex may selectively interrupt chromatin remodeling, thus not allowing for the transcription of key EMT-inducible genes.

In summary, the work presented by Venkov et al. (6) has introduced us to the CBF-A/KAP-1/FTS-1 complex, a regulator of the early events during EMT. Future studies of this protein-DNA complex will enrich our knowledge regarding the complex transcriptional cascades that facilitate EMT and provide further insights into the treatment of EMT-dependent diseases.

Acknowledgments

The research work in the laboratory of the authors is supported by the NIH (grants DK 55001, DK 62987, DK 61688, and AA 13913) and program funds provided to the Division of Matrix Biology by the Beth Israel Deaconess Medical Center. Michael Zeisberg is funded by the NIH (IK08DK074558-01) and the Carl W. Gottschalk Award 2006.

Address correspondence to: Raghu Kalluri, Harvard Medical School, Division of Matrix Biology, Beth Israel Deaconess Medical Center, 330 Brookline Ave. (Dana 514), Boston, Massachusetts 02215, USA. Phone: (617) 667-0445; Fax: (617) 975-5663; E-mail: rkalluri@bidmc.harvard.edu.

1. Kalluri, R., and Neilson, E.G. 2003. Epithelial-mesenchymal transition and its implications for fibrosis. *J. Clin. Invest.* **112**:1776–1784. doi:10.1172/JCI200320530.
2. Lee, J.M., Dedhar, S., Kalluri, R., and Thompson, E.W. 2006. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J. Cell Biol.* **172**:973–981.
3. Strutz, F. 1995. Identification and characterization of a fibroblast marker: FSP1. *J. Cell Biol.* **130**:393–405.
4. Okada, H., Danoff, T.M., Kalluri, R., and Neilson, E.G. 1997. Early role of Fsp1 in epithelial-mesenchymal transformation. *Am. J. Physiol.* **273**:F563–F574.

5. Okada, H., et al. 1998. Identification of a novel *cis*-acting element for fibroblast-specific transcription of the FSP1 gene. *Am. J. Physiol.* **275**:F306–F314.
6. Venkov, C.D., et al. 2007. A proximal activator of transcription in epithelial-mesenchymal transition. *J. Clin. Invest.* **117**:482–491. doi:10.1172/JCI29544.
7. Kamada, S., and Miwa, T. 1992. A protein binding to CArG box motifs and to single-stranded DNA functions as a transcriptional repressor. *Gene.* **119**:229–236.
8. Bemark, M., Olsson, H., Heinegard, D., and Leanderson, T. 1998. Purification and characterization of a protein binding to the SP6 kappa promoter. A potential role for CArG-box binding factor-A in kappa transcription. *J. Biol. Chem.* **273**:18881–18890.
9. Mikheev, A.M., Mikheev, S.A., Zhang, Y., Aebersold, R., and Zarbl, H. 2000. CArG binding factor A (CBF-A) is involved in transcriptional regulation of the rat Ha-ras promoter. *Nucleic Acids Res.* **28**:3762–3770.
10. Friedman, J.R., et al. 1996. KAP-1, a novel corepressor for the highly conserved KRAB repression domain. *Genes Dev.* **10**:2067–2078.
11. Schultz, D.C., Friedman, J.R., and Rauscher, F.J., 3rd. 2001. Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. *Genes Dev.* **15**:428–443.
12. Zeisberg, M., et al. 2003. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat. Med.* **9**:964–968.

To ablate or not to ablate? HSCs in the T cell driver's seat

Claudio Anasetti and James J. Mulé

H. Lee Moffitt Comprehensive Cancer Center, Tampa, Florida, USA.

The combination of the induction of lymphopenia and vaccination and/or T cell transfer is garnering much attention for cancer treatment. Preclinical studies have shown that the induction of lymphopenia by chemotherapy or radiation can enhance the antitumor efficacy of several distinct, cell-based immunotherapeutic approaches. The mechanism(s) by which such enhancement is achieved are being intensively studied, yet there is much opportunity for improvement. The animal studies reported by Wrzesinski and colleagues in this issue of the *JCI* are a promising and timely step in this direction (see the related article beginning on page 492). The authors have evaluated both the effect of increasing the intensity of lymphodepletion and the influence of HSC transfer on the in vivo function of adoptively transferred CD8$^+$ T cells. We discuss their results in light of the evolving field and their implications for advancing cell-based immunotherapies for cancer.

Current immunization approaches attempt to activate and expand the tumor-reactive T cell population in hosts with an intact immune system. There is much evidence that within the immune system of cancer patients, tumor-induced suppression and immune-based regulatory factors are present that may limit the effectiveness of vaccine-induced, tumor-specific T cells (1). An alternative approach is to induce lymphopenia (a reduction in lymphocyte number) in hosts, allowing residual host or transferred naive or antigen-specific donor T cells to undergo homeostasis-driven proliferation to restore the memory T cell compartment. Several potential advantages are offered by this strategy. For example, in addition to eliminating inhibitory immune cells in the host such as Tregs, lymphomyeloid reconstitution may overcome inherent defects in T cell signaling and may strengthen the costimulatory functions of APCs (2). Induction of lymphopenia can lead to an increased production and availability of immune response–stimulating cytokines such as IL-7 and IL-15, resulting in enhanced CD8$^+$ T cell activity (3, 4). Other studies have shown enhanced T cell trafficking into tumors after induction of lymphopenia (5, 6), as well as enhanced intratumoral proliferation of effector cells (7). It is postulated that vaccination during homeostasis-driven proliferation may serve to educate the developing T cell repertoire and lead to enhanced T cell memory against tumor-associated self antigens (8, 9).

Common methods to induce lymphopenia include treatment with low-dose total body irradiation (TBI) that produces mild, reversible myelosuppression (hence nonmyeloablative) or treatment with chemotherapeutic drugs such as cyclophosphamide (Cy) alone or in combination with fludarabine, which can induce short-term and longer-term lymphopenia in mice and humans, respectively (10). Cy-induced lymphopenia can also enhance the induction of tumor-specific CD8$^+$ T cells and can lead to protective immunity against tumors (11, 12).

In this issue of the *JCI*, Wrzesinski and colleagues (13) report on a series of animal studies undertaken to determine whether it is possible to augment adoptively transferred T cell–mediated tumor destruction by increasing the intensity of lymphodepletion. The work moves the immunotherapy field forward by demonstrating, for the first time to our knowledge, a positive influence of HSC transfer on the in vivo function of adoptively transferred CD8$^+$ T cells.

Immunotherapy in the setting of nonmyeloablative lymphodepletion

Adoptive transfer of naive or activated antigen-specific T cells immediately after induction of lymphopenia has been successful in inducing tumor regression in several murine models as well as in human clinical trials. In murine tumor models, induction of lymphopenia followed by adoptive transfer of peptide-specific T cells alone led to regression of established tumors (8). Active vaccination in combination with lymphodepletion and adoptive T cell transfer may further enhance immunity (14, 15). Studies have shown that active vaccination can skew the T cell repertoire toward self or tumor-associated antigens during homeostatic proliferation (16, 17). Hu et al. (18) have reported that tumor-specific T cells can preferentially expand in tumor vaccine–draining lymph nodes following adoptive transfer of naive

Nonstandard abbreviations used: BMT, bone marrow transplantation; MART-1, melanoma-associated antigen recognized by T cells 1; TBI, total body irradiation; T_{cm} cell, central memory T cell; T_{em} cell, effector memory T cell; TIL, tumor-infiltrating lymphocyte.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **117**:306–310 (2007). doi:10.1172/JCI30973.