Editorial

A non-coding RNA axis guides TGF-β-induced gemcitabine chemoresistance

Hardik R. Mody[1], Rajgopal Govindarajan[1,2]

[1]Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, Ohio State University, Columbus, OH, USA; [2]Translational Therapeutics, Ohio State University Comprehensive Cancer Center, Ohio State University, Columbus, OH, USA

Correspondence to: Rajgopal Govindarajan. Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, Ohio State University, Columbus, OH 43210, USA. Email: govindarajan.21@osu.edu.

Comment on: Zhuang J, Shen L, Yang L, *et al.* TGFβ1 Promotes Gemcitabine Resistance through Regulating the LncRNA-LET/NF90/miR-145 Signaling Axis in Bladder Cancer. Theranostics 2017;7:3053-67.

Submitted Oct 24, 2017. Accepted for publication Oct 31, 2017.
doi: 10.21037/tcr.2017.11.07
View this article at: http://dx.doi.org/10.21037/tcr.2017.11.07

The American Cancer Society estimates 79,030 new cases and 16,870 deaths from urinary bladder cancer (UBC) in the United States this year (1). UBC is prevalent in males and in fact is the 4th most common cancer types in males (1). Besides sex, age is another risk factor for UBC with about 90% of cases reported in the age group of 55 and above. Depending on the stage of presentation, current treatment options for UBC patients include surgery, intravesical therapy, chemotherapy, radiation therapy and immunotherapy (2,3). Chemotherapy is inevitable for advanced-staged UBC patients besides being used as a neoadjuvant or adjuvant therapy in combination with surgery and radiation therapy. However, the most common drawback of chemotherapy in UBC patients is that it often leads to tumor relapse and recurrence. While numerous reasons can be attributed to such undesirable outcomes, the concept that a small fraction of tumor cells with cancer stem cell (CSC)-like properties are able to survive the harsh chemotherapeutic regimens and able to repopulate as drug resistant tumors has been around for nearly two decades (4,5). However, therapies aimed at eradicating CSCs as an effective therapeutic strategy are not available and remains as a key focus of cancer research.

A recently published study by Zhuang *et al.* in *Theranostics* has defined the role of TGF-β/LncRNA-LET/NF90/miR-145 signaling axis in regulating gemcitabine-induced cancer stemness as well as gemcitabine resistance in UBC (6). The authors carried out *in vivo* studies in tumor xenograft model wherein UBC cell lines, namely T24 and 5637, were subcutaneously implanted in nude mice. Gemcitabine was administered in cycles (first round of doses followed by 2-week recovery period followed by 2nd and 3rd round of doses) to resemble how UBC patients on gemcitabine therapy are treated in clinics. Consistent with tumor relapses observed with gemcitabine-treated UBC patients, the authors identified that gemcitabine did not suppress tumor growth during the 2nd and 3rd round of doses likely due to the development of resistance. Consistently, gemcitabine-treated resistant UBC tumors displayed higher CSC subpopulation as evident with higher sphere formation abilities as well as elevated levels of stemness markers as compared with vehicle-treated xenografts. To further investigate the role of lncRNAs in gemcitabine-induced CSCs enrichment in UBC, the authors carried out lncRNA PCR array on gemcitabine-treated resistant xenografts. The authors identified lncRNA-LET as one of the highly downregulated lncRNA with gemcitabine treatment in resistant UBCs. Interestingly, silencing of lncRNA-LET significantly increased the tumor initiating capacity as well as primary tumor volumes *in vivo*. By conducting these studies, the authors convincingly demonstrate the role of lncRNA-LET in gemcitabine-induced cancer stemness of UBCs.

Next, the authors addressed the role of TGF-β signaling pathway in mediating gemcitabine-induced cancer stemness and chemoresistance in UBCs. Results showed significantly higher p-SMAD2 levels (activated TGF signaling pathway) in gemcitabine-treated xenografts compared with vehicle-

treated xenografts. Consistently, TGF-β1 treatment reduced lncRNA-LET levels (similar to gemcitabine treatment) in multiple UBC cell lines which was rescued by blocking the TGF-β signaling pathway (with TGFBR1 inhibitor or SMAD4 knockdown). Luciferase reporter assays confirmed the SMAD binding element (SBE) in the promoter region of lncRNA-LET and thereby provided a mechanistic basis for TGF-β1-induced lncRNA-LET downregulation in UBCs. In addition, the authors showed that forced expression of lncRNA-LET rescued TGF-β1-induced CSC-like properties including sphere forming capacities and expression of stemness markers in UBC cell lines. Overall, this set of study indicated that TGF-β induced stemness or CSC-like properties via lncRNA-LET downregulation in UBCs.

Previous studies have suggested the involvement of lncRNA-LET in NF90 protein stability regulation in hepatocellular carcinoma (7). To further examine if a similar scenario prevails in UBC, the authors determined NF90 protein levels in lncRNA-LET knocked-down and overexpressed UBCs. Knockdown of lncRNA-LET increased NF90 protein levels in UBCs while opposite effects were observed with overexpression of lncRNA-LET. The authors showed that lncRNA-LET-induced NF90 downregulation was due to elevated protein degradation with contributions from 26S proteasome system. The authors also established that lncRNA-LET effects on cancer stemness was NF90 dependent as elevated levels of CSC markers upon lncRNA-LET knockdown was reversed with simultaneous knockdown of NF90 in UBC cells. Furthermore, gemcitabine induced NF90 protein levels and that NF90 silencing counteracted gemcitabine-induced CSC enrichment as shown by CSC markers in gemcitabine-resistant UBC cells. These analyses suggested the dependency of gemcitabine- and lncRNA-LET-mediated CSC effects on NF90 regulation.

To understand the downstream effects mediated by NF90 in the regulation of cancer cell stemness, the authors carried out a microarray assay in NF90 knockdown cells. This data identified miR-145 as one of the most highly upregulated miRNAs upon NF90 knockdown in UBC cells. The authors confirmed that lncRNA-LET/NF90/miR-145 axis was functional as knockdown of lncRNA-LET reduced while overexpression of lncRNA-LET increased miR-145 levels in UBC cells. Consistently, miR-145 reduced gemcitabine-induced CSC population as well as reduced CSC markers in UBC cells. With the help of bioinformatic analyses and luciferase reporter assays, the authors further delineated HMGA2 and KLF4 (regulators of CSC) as direct targets of miR-145 in UBC. The authors demonstrated that miR-145 mediated effects against CSCs were effectively rescued by ectopic expression of HMGA2 and KLF4 establishing that lncRNA-LET/NF90/miR-145 signaling axis contribute to gemcitabine-induced cancer stemness in UBCs.

Subsequently, the authors showed that inhibiting TGF-β signaling via SMAD4 knockdown significantly reversed gemcitabine-induced CSC effects including CSC subpopulations. In addition, lncRNA-LET and miR-145, which are otherwise reduced upon gemcitabine treatment, were significantly increased with SMAD4 knockdown in gemcitabine-resistant UBC cells. Similarly, NF90 protein levels which are otherwise induced by gemcitabine, were decreased with SMAD4 knockdown in UBC cells. Finally, suppressing TGF-β signaling pathway via a TGFBR1 inhibitor (LY2157299) significantly improved anticancer effects of gemcitabine in a gemcitabine-resistant UBC xenograft mouse model. While neither gemcitabine nor LY2157299 alone showed any significant antitumor effects, however their combination treatment significantly reduced tumor volume of gemcitabine-resistant UBC xenografts. Consistently, combining LY2157299 with gemcitabine reduced CSC-like properties as well as TGF-β signaling implicating a possibility of combining gemcitabine with inhibitors of TGF-β pathway to treat UBC in clinics.

Finally, the authors demonstrated the clinical relevance of the TGF-β1/lncRNA-LET/NF90/miR-145 axis in UBC. While the expression levels of NF90 and TGF-β1 were elevated, on the other hand lncRNA-LET and miR-145 were downregulated in human UBC samples. Consistently, lncRNA-LET levels positively correlated with miR-145, both of which negatively correlated with the levels of NF90 and TGF-β1 in UBC samples. In addition, lncRNA-LET and miR-145 expressions strongly correlated with UBC patient outcomes, wherein patients with higher expressions of either survived longer. Thus, in summary, using a combination of cellular, molecular and analytical methods, the authors provided fundamental insights into how TGF-β1/lncRNA-LET/miR-145 axis can be utilized as a potential signature of outcomes in UBC patients. These findings present both diagnostic and therapeutic relevance for handling aggressive, drug resistant UBCs in patients.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Long Chen (Department of PET-CT center at the Yunnan Tumor Hospital, the Third Affiliated Hospital of Kunming Medical University, Kunming, China; Department of Biochemistry and Molecular Biology of Kunming Medical University, Kunming, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2017.11.07). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Cite this article as: Mody HR, Govindarajan R. A non-coding RNA axis guides TGF-β-induced gemcitabine chemoresistance. Transl Cancer Res 2017;6(Suppl 9):S1455-S1457. doi: 10.21037/tcr.2017.11.07

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