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RESEARCH HIGHLIGHT

The role of LMTK3 in chromatin remodeling and transcriptional regulation

Yichen Xu[1], Justin Stebbing[1], Georgios Giamas[1, 2]

[1]*Imperial College London, Division of Surgery and Cancer, ICTEM Building, W12 0NN London, UK*
[2]*School of Life Sciences, University of Sussex, Brighton BN1 9QG, UK*

Correspondence: Yichen Xu or Georgios Giamas
E-mail: y.xu11@imperial.ac.uk or g.giamas@sussex.ac.uk
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Nuclear receptor tyrosine kinases such as EGFR have been shown to be associated with increased tumor grade and poorer patient survival. One explanation for this is that following nuclear transport, these RTKs are directly involved in transcriptional regulation through chromatin binding. LMTK3 is a novel oncogenic RTK implicated in breast cancer, whose cytoplasmic and nuclear abundance are highly associated with poorer survival in breast cancer patients. So far, the function of cytoplasmic LMTK3 in breast cancer growth, invasion and endocrine resistance has been addressed, however little is known about the role of nuclear LMTK3. In our recent study, we discovered that LMTK3 binds to chromatin via its interacting partners PP1α and KAP1. Moreover, LMTK3 induces the tethering of chromatin to the nuclear periphery. These events result in chromatin condensation and subsequent transcriptional repression of various tumor suppressor-like genes, leading to breast cancer progression. Overall, this research work provides an insight of the nuclear LMTK3 function and suggests that targeting LMTK3 may have further clinical potentials in treating breast cancer.

Keywords: LMTK3; kinases; transcription; chromatin

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Introduction

Receptor tyrosine kinases (RTKs) are transmembrane proteins located at the cell surface that recognize and respond to growth factors, which results in their activation and initiation of intracellular signaling pathways. Interestingly, despite their name, RTKs are not restricted to the membrane but are routinely internalized through endocytic pathways. These kinases could either proceed to lysosomal degradation or recycle back to the plasma membrane; however, unanticipated functions can also occur, including i.e. the accumulation of the epidermal growth factor receptor (EGFR) at the endosome, which leads to autophagy initiation [1]. Interestingly, the majority of evidence indicates that the RTKs that are neither degraded nor recycled, are actually transported to the nucleus, particularly in the nucleoplasm instead of the nuclear envelope. In most cases, the presence of ligands is also essential for their nuclear transportation [2-5].

As expected, nuclear RTKs have a distinct function, which is the regulation of gene transcription. The direct involvement of EGFR in transcriptional regulation was first addressed in 2001 [2]. Noteworthy, researchers discovered the existence of nuclear EGFR in a majority of human tumors, such as skin, breast, bladder, cervix and others [6-11]. These EGFRs were highly associated with increased tumor grade and size, lymph node involvement and poorer overall survival (OS) [7, 10, 12, 13]. Chromatin immunoprecipitation (ChIP) assay demonstrated that upon EGF addition, nuclear

EGFR functions as a transcriptional co-activator on *cyclin D1* promoter, resulting in *cyclin D1* transcription activation [2]. Although no comprehensive sequencing was carried out, a few EGFR-targeted pro-tumorigenic genes have been identified, including cyclin D1 [2], iNOS [9], COX2 [14], c-Myc [15] and BCRP [16]. In addition to EGFR, a few studies have also addressed the involvement of other nuclear RTKs in transcriptional regulation; amongst them, nuclear ERBB2 can bind and regulate *cyclin D1* and *COX2* expression, acting as a co-activator of *STAT3* [17], while FGFR-I can act as a transcription factor of FGF-2 [18].

Lemur tyrosine kinase 3 (LMTK3), a member of the RTK family, was originally identified as an estrogen receptor α (ERα) regulator [19]. It is also implicated in endocrine resistance in breast cancer [20] and can promote tumor invasion and metastasis by activating the transcription of integrins [21]. Interestingly, LMTK3 is one of the few positively selected RTKs between humans and chimpanzees; considering the fact that chimpanzees have much lower susceptibility to breast cancer, this indicates the crucial role of LMTK3 in this disease [19]. Like other RTKs, LMTK3 localizes both in the nucleus and the cytoplasm and correlates with tumor grade and patients' survival [22]. Therefore, considering the involvement of certain RTKs in transcriptional regulation, we hypothesized that LMTK3 might also be able to act in a similar way by binding to chromatin.

LMTK3 represses gene transcription through chromatin binding

To validate this, we performed ChIP-sequencing to identify LMTK3 global binding events in MCF7 and MDA-MB-231 [23], which are ER^+ and ER^- breast cancer cells, respectively. After validating the antibody specificity, we confirmed that LMTK3 indeed binds to DNA both at promoter and distal regulatory regions. Interestingly, we discovered that LMTK3 binding events in both cell lines are highly similar, while LMTK3 and ERα binding events are barely correlated. In addition, anti-estrogen treatment had no effect on LMTK3 bindings. These results indicate that LMTK3 DNA binding events are ERα-independent. We then discovered that LMTK3 could bind to both active and repressive promoters; nevertheless, its bindings were more associated with repressive promoters, indicating that LMTK3 may induce transcriptional repression.

Considering the fact that LMTK3 lacks a DNA binding domain, we investigated the existence of other binding partner(s) that could mediate this effect. Combining the motif analysis and RIME assay data, we identified KAP1 as a potential binding partner of LMTK3. Interestingly, LMTK3 did not phosphorylate KAP1; on the contrary, their interaction resulted in KAP1 dephosphorylation at Ser824 through activation of phosphatase PP1α. Noteworthy, the dephosphorylation of KAP1 by LMTK3 was specific to LMTK3-associated regions, suggesting a highly specific effect. This dephosphorylation resulted in sustained chromatin condensation and transcriptional silencing. Subsequently, we also detected that LMTK3 bindings are highly associated with transcriptional silenced lamin-associated domains (LADs), the majority of which are located at the nuclear periphery. Moreover, LMTK3 is implicated in tethering LMTK3-associated regions to the nuclear periphery, inducing H3K9me3 modification and transcriptional repression.

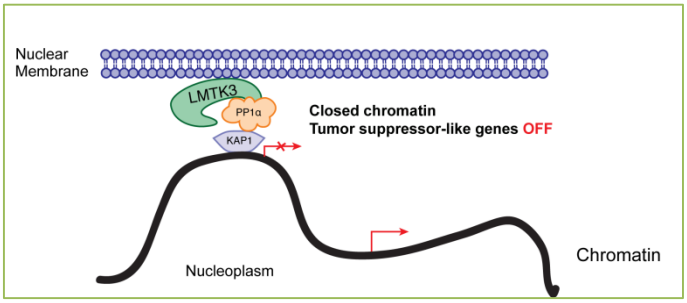

Figure 1. The model of the transcriptional co-repressor behavior of LMTK3. Graphic summary illustrating the mechanism of chromatin remodeling mediated by nuclear LMTK3. LMTK3 binds to PP1α and KAP1. As a result, KAP1 phosphorylation is suppressed. Moreover, LMTK3 tethers the whole chromatin complex to the nuclear membrane. These events result in chromatin condensation on LMTK3-associated loci and the subsequent transcriptional repression of LMTK3-bound tumor suppressor-like genes.

Finally, we observed that the majority of LMTK3-repressed genes behave like tumor suppressors that are associated with good prognosis, suggesting that the DNA binding activity of LMTK3, which suppresses a number of tumor suppressor-like genes, is important for the oncogenic role of LMTK3.

Conclusions

In aggregate, we provide evidence that LMTK3 functions as a transcriptional co-repressor through interacting with PP1α and KAP1, and as a scaffold protein by tethering the heterochromatin to the nuclear lamina, resulting in chromatin remodeling and transcriptional repression of LMTK3-bound tumor suppressor-like genes (Figure 1). However, LMTK3 also binds to active promoters, which is not mentioned in this work [23]. It is not a surprise that this distinct binding behavior is not mediated via KAP1, which is mainly found in heterochromatin and induces transcriptional repression. Therefore, other binding partners are required. One of the candidates, from our recent results, could be CREB1, which

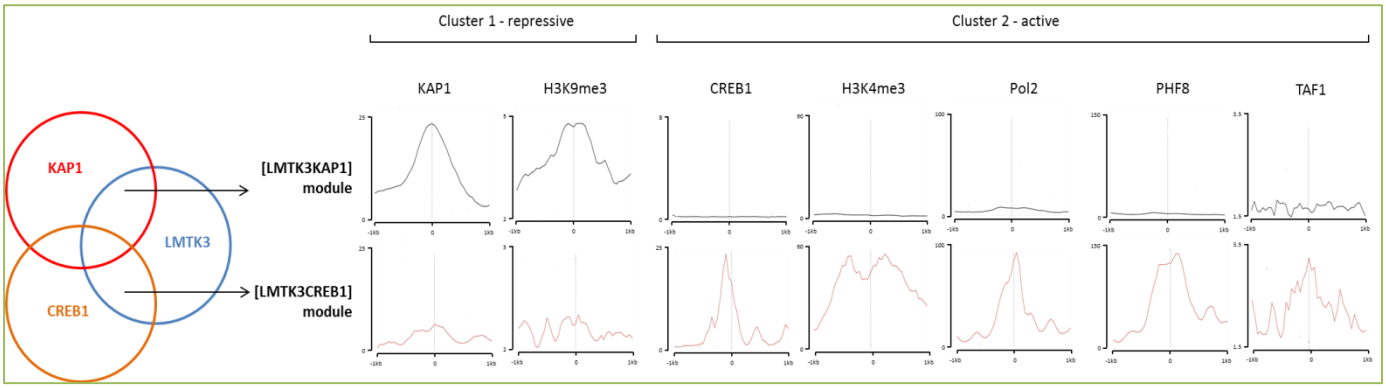

Figure 2. The [LMTK3KAP1] and [LMTK3CREB1] modules are associated with distinct combinations of chromatin regulators. Average binding profiles at regions bound by LMTK3, KAP1 and CREB1. LMTK3 and KAP1 shared regions are named [LMTK3KAP1]; LMTK3 and CREB1 shared regions are named [LMTK3CREB1]. Cluster 1 shows high binding intensity of [LMTK3KAP1], while Cluster 2 shows high binding intensity of [LMTK3CREB1].

collaborates with LMTK3 in binding to the promoter of certain oncogenes such as *PELP1*, *RPS6KB2*, *CCNA2 and PTPN11*, resulting in transcriptional activation. LMTK3-CREB1 shared binding regions are highly associated with active markers, distinct to the LMTK3-KAP1 module (Figure 2), suggesting that despite repressing a number of tumor suppressor-like genes, LMTK3 could also activate a few oncogenes using a different binding partner.

In addition, apart from the well-defined kinase domain, LMTK3 has a long C-terminal disordered domain that may participate in facilitating protein-protein interactions, implicated in a number of cellular processes, such as the chromatin binding activity described in this study. The cytoplasmic domains of the majority of RTKs are composed of their kinase domains and disordered regions. Therefore, our study suggesting that LMTK3 could act in a non-classical kinase way but instead as a scaffold able to interact with transcription factors/cofactors (i.e. PP1α, KAP1), could be applied to other RTK family members, which might give rise to the discovery of novel functions of nuclear RTKs.

Conflicting interests

The authors have declared that no competing interests exist.

References

1. Tan, X., N. Thapa, Y. Sun, and R.A. Anderson, A kinase-independent role for EGF receptor in autophagy initiation. Cell 2015; 160: 145-60.
2. Lin, S.Y., K. Makino, W.Y. Xia, A. Matin, Y. Wen, K.Y. Kwong, *et al*., Nuclear localization of EGF receptor and its potential new role as a transcription factor. Nature Cell Biology 2001; 3: 802-808.
3. Maher, P.A., Nuclear translocation of fibroblast growth factor (FGF) receptors in response to FGF-2. Journal of Cell Biology 1996; 134: 529-536.
4. Offterdinger, M., C. Schofer, K. Weipoltshammer, and T.W. Grunt, c-erbB-3: a nuclear protein in mammary epithelial cells. Journal of Cell Biology 2002; 157: 929-939.
5. Stachowiak, M.K., P.A. Maher, A. Joy, E. Mordechai, and E.K. Stachowiak, Nuclear accumulation of fibroblast growth factor receptors is regulated by multiple signals in adrenal medullary cells. Molecular Biology of the Cell 1996; 7: 1299-1317.
6. Kamio, T., K. Shigematsu, H. Sou, K. Kawai, and H. Tsuchiyama, Immunohistochemical Expression of Epidermal Growth-Factor Receptors in Human Adrenocortical Carcinoma. Human Pathology 1990; 21: 277-282.
7. Lipponen, P. and M. Eskelinen, Expression of Epidermal Growth-Factor Receptor in Bladder-Cancer as Related to Established Prognostic Factors, Oncoprotein (C-Erbb-2, P53) Expression and Long-Term Prognosis. British Journal of Cancer 1994; 69: 1120-1125.
8. Marti, U., C. Ruchti, J. Kampf, G.A. Thomas, E.D. Williams, H.J. Peter, *et al*., Nuclear localization of epidermal growth factor and epidermal growth factor receptors in human thyroid tissues. Thyroid 2001; 11: 137-145.
9. Lo, H.W., S.C. Hsu, M. Ali-Seyed, M. Gunduz, W.Y. Xia, Y.K. Wei, *et al*., Nuclear interaction of EGFR and STAT3 in the activation of the iNOS/NO pathway. Cancer Cell 2005; 7: 575-589.
10. Lo, H.W., W. Xia, Y. Wei, M. Ali-Seyed, S.F. Huang, and M.C. Hung, Novel prognostic value of nuclear epidermal growth factor receptor in breast cancer. (vol 65, pg 338, 2005). Cancer Research 2005; 65: p. 2045-2045.
11. Psyrri, A., Z.W. Yu, P.M. Weinberger, C. Sasaki, B. Haffty, R. Camp, *et al*., Quantitative determination of nuclear and cytoplasmic epidermal growth factor receptor expression in oropharyngeal squamous cell cancer by using automated quantitative analysis. Clinical Cancer Research 2005; 11:5856-5862.
12. Hadzisejdic, I., E. Mustac, N. Jonjic, M. Petkovic, and B. Grahovac, Nuclear EGFR in ductal invasive breast cancer: correlation with cyclin-D1 and prognosis. Mod Pathol 2010; 23: 392-403.
13. Xia, W.Y., Y.K. Wei, Y. Du, J.S. Liu, B. Chang, Y.L. Yu, *et al*.,

Nuclear Expression of Epidermal Growth Factor Receptor is a Novel Prognostic Value in Patients With Ovarian Cancer. Molecular Carcinogenesis 2009; 48:610-617.

14. Lo, H.W., X.Y. Cao, H. Zhu, and F. Ali-Osman, Cyclooxygenase-2 Is a Novel Transcriptional Target of the Nuclear EGFR-STAT3 and EGFRvIII-STAT3 Signaling Axes. Molecular Cancer Research 2010; 8:232-245.
15. Jaganathan, S., P. Yue, D.C. Paladino, J. Bogdanovic, Q. Huo, and J. Turkson, A functional nuclear epidermal growth factor receptor, SRC and Stat3 heteromeric complex in pancreatic cancer cells. PLoS One 2011; 6: e19605.
16. Huang, W.C., Y.J. Chen, L.Y. Li, Y.L. Wei, S.C. Hsu, S.L. Tsai, *et al*., Nuclear Translocation of Epidermal Growth Factor Receptor by Akt-dependent Phosphorylation Enhances Breast Cancer-resistant Protein Expression in Gefitinib-resistant Cells. Journal of Biological Chemistry 2011; 286:20558-20568.
17. Beguelin, W., M.C.D. Flaque, C.J. Proietti, F. Cayrol, M.A. Rivas, M. Tkach, *et al*., Progesterone Receptor Induces ErbB-2 Nuclear Translocation To Promote Breast Cancer Growth via a Novel Transcriptional Effect: ErbB-2 Function as a Coactivator of Stat3. Molecular and Cellular Biology 2010; 30:5456-5472.
18. Peng, H., J. Moffett, J. Myers, X. Fang, E.K. Stachowiak, P. Maher, *et al*., Novel nuclear signaling pathway mediates activation of fibroblast growth factor-2 gene by type 1 and type 2 angiotensin II receptors. Mol Biol Cell 2001; 12:449-62.
19. Giamas, G., A. Filipovic, J. Jacob, W. Messier, H. Zhang, D. Yang, *et al*., Kinome screening for regulators of the estrogen receptor identifies LMTK3 as a new therapeutic target in breast cancer. Nat Med 2011; 17:715-9.
20. Stebbing, J., A. Filipovic, L.C. Lit, K. Blighe, A. Grothey, Y. Xu, *et al*., LMTK3 is implicated in endocrine resistance via multiple signaling pathways. Oncogene 2013; 32:3371-80.
21. Xu, Y., H. Zhang, L.C. Lit, A. Grothey, M. Athanasiadou, M. Kiritsi, *et al*., The kinase LMTK3 promotes invasion in breast cancer through GRB2-mediated induction of integrin beta(1). Sci Signal 2014; 7: ra58.
22. Stebbing, J., A. Filipovic, I.O. Ellis, A.R. Green, T.R. D'Silva, H.J. Lenz, *et al*., LMTK3 expression in breast cancer: association with tumor phenotype and clinical outcome. Breast Cancer Res Treat 2012; 132:537-44.
23. Xu, Y., H. Zhang, V.T. Nguyen, N. Angelopoulos, J. Nunes, A. Reid, *et al*., LMTK3 Represses Tumor Suppressor-like Genes through Chromatin Remodeling in Breast Cancer. Cell Rep 2015; 12:837-49.