IncRNA LINC-PINT is downregulated in melanoma and regulates cell proliferation by downregulating IncRNA BANCR

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Received December 24, 2018; Accepted June 13, 2019

DOI: 10.3892/ol.2019.10631

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Key words: melanoma, long non-coding RNA LINC-PINT, long non-coding BANCR, proliferation

Abstract. The development of melanoma may involve long non-coding RNAs (lncRNAs); however, the functions of the majority of lncRNAs in melanoma are unknown. The present study investigated the role of long intergenic non-protein coding RNA p53 induced transcript (LINC-PINT) in melanoma. In the present study, quantitative PCR was used to detect gene expression, overexpression experiments were performed to analyze gene interactions and CCK-8 assays were used to analyze cell proliferation. LINC-PINT was downregulated, while BRAF-activated non-coding RNA (BANCR) was upregulated in melanoma tissues compared with normal adjacent tissues. Expression levels of LINC-PINT decreased, while expression levels of BANCR increased with increasing tumor thickness. The expression levels of LINC-PINT and BANCR were inversely associated in melanoma tissues but not in healthy adjacent tissue. LINC-PINT overexpression downregulated BANCR expression in melanoma cells, while BANCR overexpression did not significantly affect LINC-PINT expression. LINC-PINT overexpression inhibited melanoma cell proliferation in vitro compared to controls. BANCR overexpression attenuated the effects of LINC-PINT overexpression. The present study revealed that IncRNA LINC-PINT is downregulated in melanoma and may regulate melanoma cell proliferation by downregulating IncRNA BANCR.

Introduction

As the most aggressive skin cancer, melanoma is characterized by the rapid progression (1). Surgical resection of primary tumors usually results in satisfactory outcomes for patients at early stages (2). However, the development of melanoma is usually accompanied by tumor metastasis to regional lymph nodes or even distant organs, which lacks radical treatment (3). At present, the 5-year survival rate of metastatic melanoma patients remains <20% (4). The unclear pathogenesis of melanoma is the major challenge for clinical treatment of this disease (5). Identification of novel therapeutic targets is always needed to improve the survival of melanoma patients.

Long non-coding RNAs (lncRNAs) are RNA transcripts consisting of >200 nucleotides with no protein-coding capacity (6). Different from messenger RNAs, lncRNAs participate in cellular processes by regulating gene expression at post-transcriptional and translational levels, or even through epigenetic pathways (6,7). There is mounting evidence that lncRNAs are critical determinants in human diseases, and dysregulated lncRNA expression is closely correlated with the occurrence of many cancers (8). Regulation of lncRNA expression has been proven as potential therapeutic target for cancer treatment (9). However, function of most lncRNAs remains unclear. LncRNA LINC-PINT is a recently identified tumor suppressor in different types of cancer, such as lymphoblastic leukemia (10,11). In the present study we investigate the involvement of LINC-PINT in melanoma, and explored its interactions with BANCR, which promotes melanoma (12).

Materials and methods

Research subjects. A total of 60 patients with melanoma [35 males and 25 females; age range, 28 to 69 years; mean age, 49.7±5.6 (standard deviation) years] were enrolled in Chongqing Traditional Chinese Medicine Hospital (Chongqing, China) between January 2015 and January 2018. All patients were diagnosed pathologically by at least 3 experienced pathologists. The inclusion criteria for enrolment in the current study were as follows: i) Patients with melanoma patients with no history of other malignancies; and ii) patients willing to
participate. The exclusion criteria were as follows: i) Patients complicated with other skin diseases or other severe diseases, including other types of cancer; and ii) patients who had been treated within 3 months prior to admission. According to the American Joint Committee on Cancer staging (13), there were 23, 18 and 19 cases at stage I, II and III, respectively. According to the thickness of primary tumors, there were 15 cases <1 mm, 16 cases between 1-2 mm, 15 cases between 2-4 mm and 14 cases >4 mm. The current study was approved by the Ethics Committee of Chongqing Traditional Chinese Medicine Hospital (Chongqing, China). All patients signed informed consent.

**Specimens and cell lines.** Tumor tissues and adjacent (within 2 cm around tumors) healthy tissues were collected through biopsy and were stored in a liquid nitrogen sink at -196˚C prior to use. Tissues were stored in liquid nitrogen prior to use. The melanoma cell lines A375-P and A375-MA2 (ATCC; American Type Culture Collection, Manassas, VA, USA) were used in the current study. Cells were cultured using ATCC-formulated Dulbecco's Modified Eagle Medium (American Type Culture Collection) containing 10% fetal bovine serum (American Type Culture Collection) in an incubator at 37˚C and 5% CO₂.

**Total RNA extraction and reverse-transcription quantitative polymerase chain reaction (RT-qPCR).** RNAzol® reagent (GeneCopoeia, Inc., Rockville, MD, USA) was used to extract total RNA from tissue specimens and *in vitro* cultured A375-P and A375-MA2 cells. Tissues were ground in liquid nitrogen prior to the addition of RNAzol® reagent. A RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to synthesize cDNA through reverse transcription using the following conditions: 25˚C for 5 min, 55˚C for 30 min and 80˚C for 15 min. To detect the expression of LINC-PINT and BANCR, SYBR™-Green Master mix (Thermo Fisher Scientific, Inc.) was used to prepare all PCR reaction systems. CFX96 Touch™ Real-Time PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to perform all PCR reactions with 18S RNA as endogenous control. Primer sequences were as follows: LINC-PINT, forward, 5'-CGT GGG AGC CCC TTT CGT-3' and reverse, 5'-ATG AAG AAA GCC TGG TGC AGT-3'; TC-3'; BANCR forward, 5'-ACA GGA CTC CAT GGC AAA-3' and reverse, 5'-AGC TAT CAA TCT GTC AAT CCT GTC-3'.

**Statistical analysis.** Three biological replicates were performed for each experiment. GraphPad Prism software (version 6; GraphPad Software, Inc., La Jolla, CA, USA) was used to process the data and perform statistical analysis. Data are expressed as the mean ± standard deviation. Comparisons of expression levels of LINC-PINT and BANCR among the tumor thickness groups, as well as comparisons of expression levels of LINC-PINT and BANCR and cell proliferation data among cell groups were performed by one-way ANOVA followed by a Tukey post hoc test. Associations between LINC-PINT and BANCR were analyzed by linear regression. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Expression of LINC-PINT and BANCR is altered in melanoma tissues.** The expression levels of LINC-PINT and BANCR in 60 patients with melanoma were detected by RT-qPCR. Compared with healthy adjacent tissues, LINC-PINT was significantly downregulated in tumor tissues (Fig. 1A; P<0.05). By contrast, BANCR was significantly upregulated in tumor tissues compared with healthy adjacent tissues (Fig. 1B; P<0.05).

**Expression of LINC-PINT and BANCR is affected by tumor thickness.** Primary tumors were classified based on thickness. There were 15 cases <1 mm, 16 cases between 1-2 mm, 15 cases between 2-4 mm and 14 cases >4 mm. The expression levels of LINC-PINT significantly decreased (Fig. 2A; P<0.05), while the expression levels of BANCR significantly increased (Fig. 2B; P<0.05) with increasing tumor thickness. **LINC-PINT and BANCR are inversely associated.** Associations between LINC-PINT and BANCR were analyzed by linear regression. The expression levels of LINC-PINT and BANCR were significantly and inversely associated in melanoma tissues (Fig. 3A; P<0.01). However, LINC-PINT and BANCR expression levels were not significantly associated in healthy adjacent tissues (Fig. 3B; P=0.57).
LINC-PINT is a likely upstream inhibitor of BANCR in melanoma cells. The significantly inverse association between LINC-PINT and BANCR in tumor tissues indicated the possible interactions between LINC-PINT and BANCR. To further investigate the interaction between LINC-PINT and BANCR, vectors expressing LINC-PINT and BANCR were transfected into A375-P and A375-MA2 melanoma cell lines. Overexpression in A375-P and A375-MA2 cells was achieved 24 h following transfection and compared with untransfected cells (C group) and cells transfected with empty vectors (NC group; Fig. 4A; P<0.05). Compared with the C and NC groups, cells overexpressing LINC-PINT revealed significantly downregulated BANCR levels (Fig. 4B; P<0.05), while cells with BANCR overexpression revealed no significant changes in the LINC-PINT expression level compared with the C and NC groups (Fig. 4C; P>0.05).

LINC-PINT overexpression inhibits melanoma cell proliferation through BANCR. LINC-PINT and BANCR expression levels were significant increased following co-transfection with LINC-PINT and BANCR expression vectors compared with the C and NC groups (Fig. 5A; P<0.05). Compared with untransfected cells (C group) and cells transfected with empty vectors (NC group), cell proliferation was decreased in cells overexpressing LINC-PINT at 96 h (Fig. 5B; P<0.05). BANCR overexpression increased proliferation compared with the C and NC groups (Fig. 5B; P<0.05). Co-transfection with LINC-PINT and BANCR expression vectors attenuated the effects of LINC-PINT overexpression (Fig. 5B; P<0.05).

Discussion

LINC-PINT is a recently identified tumor suppressor in different types of cancer, including retinoblastoma and gastric cancer (10,11); however, its role in melanoma remains unknown. The current study, to the best of our knowledge, was the first to show the downregulated expression pattern of LINC-PINT in melanoma, and suggested that LINC-PINT may be a tumor suppressor in this disease. Furthermore, the current study demonstrated that the actions of LINC-PINT in melanoma are likely achieved through the interaction with BANCR.

BANCR is a well-characterized oncogenic lncRNA in different types of cancer, including retinoblastoma and gastric cancer (12,15,16). Upregulation of BANCR promoted tumor...
growth and metastasis, and indicated poor survival of patients with retinoblastoma and gastric cancer (15,16). Li et al (12) demonstrated that BANCR promoted cancer cell proliferation in malignant melanoma. Consistent with the aforementioned result, the current study revealed upregulated expression of BANCR in melanoma tissues compared with healthy adjacent tissues. Furthermore, overexpression of BANCR promoted proliferation of melanoma cells in vitro. The results obtained in the current study further demonstrated the oncogenic roles of BANCR in melanoma.

The oncogenic or tumor suppression roles of lncRNAs are achieved through the interactions with downstream tumor suppression or oncogenic pathways (17,18). Previous studies have revealed that lncRNAs may interact with other non-coding RNAs, including microRNAs, to participate in cancer biology (17-19). However, studies on the interactions between different lncRNAs are rare. The present study revealed that LINC-PINT is downregulated in melanoma tissues compared with healthy adjacent tissues and may serve a role as tumor suppressor in this disease. Furthermore, the present study suggested that LINC-PINT may exert its effects in melanoma by serving as an upstream inhibitor of BANCR. BANCR has previously been revealed to activate the mitogen-activated protein kinase (MAPK) signaling pathway to promote the development of melanoma (12). A previous study
demonstrated that LINC-PINT interacts with MAPK in acute myocardial infarction (20). Additionally, BANCR has been reported to interact with MAPK (13). Therefore, LINC-PINT may interact the BANCR/MAPK signaling pathway to inhibit melanoma cell proliferation, and MAPK may mediate the interaction between LINC-PINT and BANCR. However, the current study did not investigate the role of MAPK. Future studies are required to elucidate the role of MAPK in the interaction between LINC-PINT and BANCR. The results obtained in the current study enriched the understanding of the molecular mechanisms in melanoma.

Notably, LINC-PINT overexpression failed to significantly affect the migration and invasion of melanoma cells (data not shown). Therefore, LINC-PINT may specifically inhibit the proliferation, but not other behaviors, of melanoma cells.

In conclusion, LINC-PINT is downregulated in melanoma, and LINC-PINT overexpression may inhibit melanoma cell proliferation by downregulating BANCR. The current study suggested that LINC-PINT may serve as a potential therapeutic target for melanoma.

Acknowledgements
Not applicable.

Funding
No funding was received.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
ML designed the study. QH, QD and DZ performed all the experiments, analyzed the data and were major contributors in writing the manuscript. All authors read and approved the final manuscript.
Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of Chongqing Traditional Chinese Medicine Hospital. All the patients provided written informed consent for participation in this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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