Editorial

The non-coding control of cancer cell proliferation: the role of lncRNA AB074169 in papillary thyroid carcinoma

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Comment on: Gou Q, Gao L, Nie X, *et al.* Long Noncoding RNA AB074169 Inhibits Cell Proliferation via Modulation of KHSRP-Mediated CDKN1a Expression in Papillary Thyroid Carcinoma. Cancer Res 2018;78:4163-74.

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Human thyroid carcinomas deriving from the follicular cell are commonly divided into three categories based on different clinical-pathological parameters: (I) well differentiated, (II) poorly differentiated and (III) undifferentiated thyroid carcinomas. Well-differentiated thyroid carcinomas include papillary thyroid carcinomas (PTC) and follicular thyroid carcinomas (1,2). PTC represents the main histotype of thyroid cancer (approximately 80% of all thyroid tumors). It is a well-differentiated tumor, but it can nevertheless slowly lead to aggressive, metastasizing and lethal forms (1). In fact, despite its good degree of differentiation, in recent years it has been seen how it is becoming a major problem for human health (3,4). Several therapeutic approaches are performed nowadays in clinical practice; surgical resection and treatment with radioactive iodine represent efficient treatments currently applied in clinical practice (3,5). Nevertheless, a major problem is that PTC patients are often diagnosed only late, and this limits the applicable treatment options, leading to a general worsening of the prognosis. Therefore, this scenario highlights the urgent need to develop new rapid and sensitive screening methodologies for the diagnosis of PTC and to clarify the molecular mechanisms on the basis of this disease.

In the latest years the scientific world is focusing on the characterization of the role played by non-coding RNA (ncRNA) molecules in the process of human tumorigenesis and, among them, by long non-coding RNAs (lncRNAs) (6). LncRNAs constitute a novel class of ncRNA that is not fully characterized yet. Scientists have now confirmed that these molecules are as long as 200 bp up to 100,000 bp and do not have open reading frames within their sequence. However, even if they lack any coding capacity, it has been observed that they play key roles in multiple biological processes, including regulation of gene expression, cell cycle and differentiation (7). Furthermore, recent studies have demonstrated their involvement in cancer showing how their up- or down-regulation might affect the modulation of several effector genes and, therefore, specific biological processes (8). Moreover, their specific deregulation in human cancers of different histotypes lead to consider lncRNAs as potential diagnostic and prognostic markers, thus giving a strong support in the early diagnosis and the monitoring of tumor disease. Even though evidences suggest that lncRNAs are involved in the neoplastic transformation of the thyroid gland, little is still known about their exact action mechanisms. Indeed, studies carried out in recent years have reported the deregulated expression of several lncRNAs in PTC. In particular, it has been observed that many of these lncRNAs aberrantly expressed in the PTC are directly involved in the control of cell growth, migration and invasion (9,10), suggesting their fundamental role in tumor progression. Furthermore, it is worth noting that some of these lncRNAs deregulated in PTC have a meaningful role of tumor suppressor and growth inhibitor (11-13), suggesting their involvement in the mechanisms that block tumor onset. In this context, it is of great interest the paper published by Gou *et al.* on *Cancer Research* (14) focusing on the molecular mechanism acted by AB074169 in PTC.

The authors found that the expression of the lncRNA AB074169 resulted down-regulated in PTC tissues if compared with their adjacent tissues, through the use of a high-throughput microarray analysis. They found that this lncRNA was 11.78-fold less expressed in tumors, with respect to the control. Subsequently, through the use of qRT-PCR on a much larger series of PTC (n=47 cases) they confirmed that AB074169 was less expressed in tumor tissues compared to adjacent non-pathological tissues. The diminished expression of AB074169 in PTC samples immediately suggests its possible role in thyroid tumorigenesis and, especially, in cell proliferation. In fact, the involvement of AB074169 in PTC development was interestingly confirmed by the correlation of its expression with patients' clinical-pathological features. In particular, a positive association was observed between high expression of AB074169 and small tumor size, thus highlighting the role of AB074169 in the control of cell proliferation during thyroid tumorigenesis. Furthermore, the involvement of AB074169 occurs very early since its decreased expression was already found in stage I/II carcinomas.

This lncRNA has not been precisely characterized and described yet in the scientific literature, but Gou *et al.* have begun to report its features. Through the use of the RACE technique, Gou *et al.* identified the existence of two isoforms of AB074169 called AF1 and AF2. However, they found that both in the cultured cell lines and in the tumor tissues of the patients the main isoform is the shortest one (AF1). Furthermore, they observed that the localization of AB074169 within the cell is cytosolic. This observation is of primary importance to explain the subsequent AB074169 functional mechanism.

Different regulatory mechanisms may contribute to repress gene expression and, among them, promoter hypermethylation in PTC is a very common phenomenon that affects tumor suppressor genes (15). Moreover, it has been reported that, similar to other coding genes, also lncRNAs undergo transcriptional repression through hypermethylation of the CpG islands (16). For this reason Gou *et al.* analyzed the AB074169 promoter region in cultured cell lines obtained from PTC and demonstrated that one of the mechanisms driving the down-regulation of AB074169 in PTC is the hypermethylation of the CpG islands within its promoter. In particular, 6 CpG islands located in a region of 1.5 kb upstream of the transcription start site resulted hypermethylated.

The reduction of AB074169 expression observed in PTC is directly reflected in a proliferative advantage of tumor cells that, consequently, gain the ability of expand and overrun the thyroid gland. *In vitro* and *in vivo* experiments described in the paper clearly reported that the restoration of AB074169 expression in thyroid carcinoma cells immediately leads to cell cycle arrest with the concomitant tumor growth inhibition. In particular, *in vitro* overexpression experiments performed by Gou *et al.* on PTC-derived cell lines (BCPAP and TPC-1) have shown that AB074169 is able to reduce cell proliferation, repress the ability to form colonies, inhibit the replication of DNA and, finally, block cells in the G2/M phase of the cell cycle. Experiments carried out *in vivo* through the generation of xenograft from BCPAP and TPC-1 overexpressing AB074169, similarly confirmed the crucial role of AB074169 since tumors formed in nude mice are characterized by lower volume, lower weight, and the decrease of the Ki67 proliferative index, in comparison to AB074169 non-overexpressing controls.

The mechanism through which AB074169 exerts its function is very interesting, as it sheds light on one of the little-known aspects of the lncRNA function, that is the regulation of downstream genes. Indeed, from the transcriptomic analysis of BCPAP cells in which AB074169 was re-expressed, it is evident that a vast array of proteins involved in the control of DNA replication and cell cycle pathways were modified. While p21 was found to be increased, CDK1, CDK2 and PCNA were found to be decreased after the overexpression of AB074169. Gou *et al.* also reported a positive correlation between expression levels of AB074169 and p21 in patients affected by PTC. Interestingly, this could explain why tumors that maintain the expression of AB074169 show, in general, a lower proliferative index. This finding further confirms that the expression of p21 is regulated by a system involving multiple lncRNAs (including AB074169) as also reported in additional scientific works (17,18).

In an attempt to explain how this kind of regulation could happen, and since it is known that lncRNAs are able to interact with RNA binding proteins (19), Gou *et al.* then performed an RNA-pulldown assay with the aim to identify protein interactors. By using this approach, followed by the identification of co-precipitated proteins by mass spectrometry, they identified KHSRP as a specific AB074169 interactor in Nthy-ori 3-1 thyroid cells. As expected, Gou *et al.* identified an RNA-interacting protein, which further confirms that lncRNAs perform their

function through the binding with protein partners (20). Subsequently, the interaction between AB074169 and KHSRP was confirmed by approaches such as RIP, while immunofluorescence co-localization experiments demonstrated that this interaction is specific and takes place in the cytoplasm.

From the scientific literature it is known that the KHSRP protein is able to bind the ARE elements (sequences rich in AU), in the 3'-UTR region of the target messenger RNAs (including the mRNA of p21) destabilizing them and promoting their degradation (21,22).

The interaction between AB074169 and KHSRP is very important considering that KHSRP is a proliferative and pro-tumorigenic factor (23). Therefore, this observation spontaneously raises the question of whether KHSRP is directly involved in the regulation mechanism performed by AB074169 on p21. Gou *et al.* responded very brightly to this question, demonstrating that KHSRP binds the mRNA of p21 (regardless of the presence or absence of AB074169), and attenuates the increase of p21 expression when AB074169 is expressed, confirming that KHSRP plays a central role in the whole mechanism.

This discovery further confirms the fundamental role that lncRNAs play in the regulation of gene expression through interaction with RNA-binding proteins, and, more in detail, with KHSRP. A few years ago, in fact, it was observed that lncRNA H19 is able to bind KHSRP in the cytoplasm of mouse mesenchymal cells C2C12 promoting the degradation of the myogenin mRNA by KHSRP and maintaining the undifferentiated state of C2C12 cells (24). The interaction between lncRNA H19 and KHSRP is in turn blocked by the induction of AKT which, consequently, re-establishes myogenin expression levels, thus supporting differentiation. It is interesting to note that lncRNA H19 seems to act as a scaffold to favor the functioning of KHSRP on the myogenin mRNA (24). Most likely, in this type of mechanism the sequence of the lncRNA that interacts with the RNA binding proteins, could direct these latters towards the selection of particular mRNAs (personal observation).

The proposed mechanism is very interesting as it allows to explain the cell cycle arrest mediated by AB074169. The central node would be the induction of p21 by AB074169, with the subsequent repression of CDK2 made by p21 itself. It is worth noting that the authors confirmed the validity of this mechanism by using several experimental approaches. However, the presence of KHSRP makes the mechanism even more complex and intricate. In fact, Gou *et al.* also demonstrated that AB074169 is able to repress KHSRP expression through a post-transcriptional mechanism driven by their interaction that finally leads to the reduction of the KHSRP mRNA half-life *in vitro*. Moreover, *in vivo* experiments showed a negative correlation between the expression levels of AB074169 and those of KHSRP.

Finally, by performing RIP experiments, Gou *et al.* showed how the STAU1 protein, responsible for the degradation of certain mRNAs, is associated with both AB074169 and KHSRP. STAU1 protein is known to be responsible of regulating the decay of several mRNAs through a mechanism named STAU1-mediated mRNA decay (SMD) (25). Therefore, AB074169 interacts with the KHSRP mRNA and promotes its destabilization through the STAU1 protein complex. As a result, since its reduced expression, KHSRP is no longer able to repress p21, this resulting in cell cycle arrest.

In conclusion, in this paper Gou *et al.* show the dual ability of AB074169 to bind the KHSRP protein and mRNA, reducing its expression and, therefore, preventing its function. As a consequence, the decreased expression of KHSRP allows p21 to increase and CDK2 to decrease, thus explaining cell cycle arrest and growth inhibition observed in PTC-derived cell lines. Looking at thyroid tumorigenesis, the mechanism explains how starting from the hypermethylation of its promoter, the AB074169 expression is switched off with a consequent overexpression of the KHSRP protein. This latter, finally, binds p21 mRNA thus inducing its degradation and promoting CDK2 increase which, in turn, stimulates cancer cell proliferation.

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Footnote

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