Meta Gene 25 (2020) 100748

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

PTEN-induced putative kinase 1 (PINK1) down-regulation in breast cancer samples in association with mitotic rate

Hajar Yaghoobi[a], Hakim Azizi[b], Vahid Kholghi Oskooei[c], Mohammad Taheri[d,*], Soudeh Ghafouri-Fard[e,*]

[a] Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[b] Department of Medical Parasitology, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran
[c] Department of Laboratory Sciences, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran
[d] Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
PINK1
PINK1-AS
Breast cancer

ABSTRACT

PINK1 codes for a serine/threonine-protein kinase located in the mitochondria. This protein contributes in the pathophysiology of both neurodegenerative diseases and cancer. Its transcription has been shown to be regulated by a natural occurring antisense (AS) RNA named *PINK1-AS*. We examined expression levels of *PINK1* and *PINK1-AS* in 54 breast cancer specimens versus their nearby non-cancerous tissues. We found significant down-regulation of *PINK1* in tumoral tissues compared with nearby tissues (P = .003). Yet, expression of *PINK1-AS* was not remarkably different between tumoral tissues and nearby tissues. Relative expression of *PINK1* was associated with mitotic rate (P = .03). We also found trends toward over-expression of *PINK1* in younger patient compared with older patients (P = .09) and in grade 2 tumors compared with grade 3 ones (P = .08). The current study delivers further evidences for contribution of *PINK1* in the pathophysiology of breast cancer and highlights the context-dependent properties the encoded protein.

1. Introduction

PTEN-induced putative kinase 1 (*PINK1*) codes for a serine/threonine-protein kinase which is located in the cell mitochondria (Unoki and Nakamura, 2001). The protein has prosurvival role at neuronal mitochondria. Germline mutations within this gene have been linked with heritable Parkinson's disease which is manifested in young persons (Berthier et al., 2011). Moreover, *PINK1* over-expression has been detected in cancer cell lines with higher metastatic capability. Notably, its sequence homology with the BRCA1-binding protein (BRAP2) (Nakajima et al., 2003) implies its contribution in BRCA1 related pathways. In silico assessment of cancer datasets has shown PINK1 over-expression in a fraction of kidney, endometrial, blood and parathyroid cancers despite its down-regulation in numerous other cancers such as ovarian and liver carcinomas. Such different patterns of *PINK1* expression between human malignancies suggest that PINK1 might have a dual anti- and pro-tumorigenic capacities based on the tumor environment (O'Flanagan and O'Neill, 2014). The *PINK1* gene resides on chromosome 1p36 (Valente et al., 2001), in a location which is repeatedly lost in a wide spectrum of malignancies and has been suggested to contain one or several tumor suppressors (Bagchi and Mills, 2008). A previous study has demonstrated cytoplasmic diffuse PINK1 expression as well as a noticeable membrane expression in breast carcinoma cells in contrast to the granular cytoplasmic pattern and the low membrane expression detected in normal breast tissue specimens (Berthier et al., 2011). In MCF-7 breast cancer cells, PINK1 has anti-apoptotic and growth-suppressive functions (Berthier et al., 2011). The observed anti-apoptotic effects of PINK1 in MCF-7 is in contrast with the most noticeable property of *PINK1* as a gene whose expression is triggered by up-regulation of *PTEN* (Unoki and Nakamura, 2001) and down-regulates the PI3-kinase (PI3K)/Akt signaling. However, despite these characteristics, overexpression of the *PINK1* transcript did not suppress cell growth in some studied cancer cell lines (Unoki and Nakamura, 2001). *PINK1-antisense* (*PINK1-AS*) is a naturally occurring non-coding antisense (NAT) which has a nucleotide sequence homology to the region that encodes C-terminus regulatory domain of PINK1. This NAT has been shown to positively regulate the quantity of its cis-transcribed mRNA under normal conditions (Scheele et al., 2007). According to the proposed roles for NATs in the modulation of gene expression and their participation in a variety of cancers (Nikpayam et al.,

* Corresponding authors.
E-mail addresses: mohammad_823@yahoo.com (M. Taheri), s.ghafourifard@sbmu.ac.ir (S. Ghafouri-Fard).

https://doi.org/10.1016/j.mgene.2020.100748
Received 12 May 2020; Received in revised form 3 June 2020; Accepted 11 June 2020
Available online 13 June 2020

2017), and the putative role of *PINK1* in the pathogenesis of breast cancer, we aimed to assess expression of *PINK1* and *PINK1-AS* in breast cancer samples compared with their nearby non-cancerous tissues.

2. Material and methods

2.1. Samples

We performed expression analysis on 54 breast cancer samples and their paired nearby tissues. All patients had been diagnosed to have invasive ductal carcinoma. Subjects were diagnosed using the criteria provided by World Health Organization (Sinn and Kreipe, 2013). Specimens were gathered from Sina and Farmanieh Hospitals during 2018, snap frozen in liquid Nitrogen and carried to Department of Medical Genetics for expression analysis. All patients were newly diagnosed patients. No patients had taken any treatment such as neoadjuvant chemotherapy or radiotherapy before surgical excision. All patients signed the informed consent forms. The study protocol was approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences. Hormone receptors and HER2 status have been gauged by immunohistochemistry strategy based on the guidelines and recommendations provided by the American Society of Clinical Oncology/College of American Pathologists (Wolff et al., 2007; Hammond et al., 2010). Nearby tissues were confirmed to be devoid of the malignant cells by the expert specialist.

2.2. Expression analysis

RNA extraction and cDNA synthesis were perfomed by using the TRIzol™ material (Invitrogen, Carlsbad, CA, USA) and cDNA Synthesis Kit (TaKaRa, Japan), respectively. Transcript levels of *PINK1* and *PINK1-AS* genes were measured in tumoral tissues versus nearby tissues using TaqMan Master Mix (Applied Biosystems). All experiments were executed in the rotor gene 6000 Corbett System in duplicate. Expressions of *PINK1* and its NAT were normalized with the expression of the housekeeping gene *Hypoxanthine-guanine phosphoribosyl transferase 1* (*HPRT1*). The information about primers and probes are displayed in Table 1. PCR efficiency and threshold cycle (Ct) quantities were used for quantification of RNA levels of each gene in tumoral tissues and nearby tissues.

2.3. Statistical analysis

Statistics were investigated using the Statistical Package for the Social Sciences (SPSS) v.20.0 (SPSS Inc., Chicago). The association between clinical aspects and relative expression of *PINK1* and its NAT was evaluated using Chi-square. Tukey's honest significance test was applied to investigate the variance between means of RNA levels between different sets of cases. Fold changes of expressions were quantified using the efficiency adjusted strategies. Correlation between relative transcripts levels of *PINK1* and its NAT was weighed using the regression model. The level of significance was judged at $P < .05$. The receiver operating characteristic (ROC) curve was depicted to estimate the properness of transcript amounts for discriminating tumoral versus nearby tissues.

Table 2
Overall aspects of enrolled patients (ER: estrogen receptor, PR: progesterone receptor).

Variables	Values
Age (years) (mean ± SD)	51.79 ± 13.54 (29–81)
Menarche age (years) (mean ± SD)	13 ± 1.65 (10–18)
Menopause age (years) (mean ± SD)	44.91 ± 14.91 (38–60)
Primary pregnancy age (years) (mean ± SD)	18.04 ± 8.36 (14–32)
Breast feeding length (months) (mean ± SD)	41.62 ± 34.1 (3 − 120)
Positive family history for other cancers (%)	17%
Cancer stage (%)	
I	30.8
II	28.8
III	30.8
IV	9.6
Grade (%)	
I	17
II	49
III	34
Mitotic rate (%)	
I	45.2
II	42.9
III	11.9
Maximum Tumor zdimention (%)	
< 2 cm	32
≥2 cm, < 5 cm	66
≥5 cm	2
ER (%)	
Positive	87.8
Negative	12.2
PR (%)	
Positive	77.1
Negative	22.9
Her2/neu expression (%)	
Positive	25
Negative	75

3. Results

3.1. Overall aspects of breast cancer patients

Overall aspects of enrolled patients are demonstrated in Table 2.

3.2. Relative expression of PINK1 and PINK1-AS in breast cancer specimens versus nearby tissues

PINK1 expression was meaningfully lower in malinant samples compared with nearby tissues (Fold change = 0.19, $P = .003$). However, expression of *PINK-AS1* was not different between tumoral tissues and nearby tissues (Fold change = 0.64, $P = .36$). Fig. 1 displays the relative expression of *PINK1* and its NAT in malignant tissues and nearby tissues as designated by –delta Ct amounts ($CT_{\text{reference gene}}$-

Table 1
The nucleotide sequences of primers and probes used for assessment of expression of *PINK1* and *PINK1-AS* genes.

Gene	Nucleotide sequence of primers/ probes	Length of primers/ probes	Length of amplicon
HPRT1	F: AGCCTAAGATGAGAGTTC	18	88
	R: CACAGAACTAGAACATTGATA	21	
	FAM -CATCTGGAGTCCTATTGACATCGC- TAMRA	24	
PINK1	F: GGAGTATGGAGCAGTCACTTACAG	24	111
	R: AGCAGCGGCACGGAAGAG	18	
	FAM-ACATCATCCGGGTTCTCCGCGCCT-TAMRA	24	
PINK1-AS	F: GGTCCACGCCTTCCAGCAG	19	165
	R: TTCCTCGCATCTCCTGTTCCTG	20	
	FAM - CCGCCTCGCCGCCATGATGCTG -TAMRA	22	

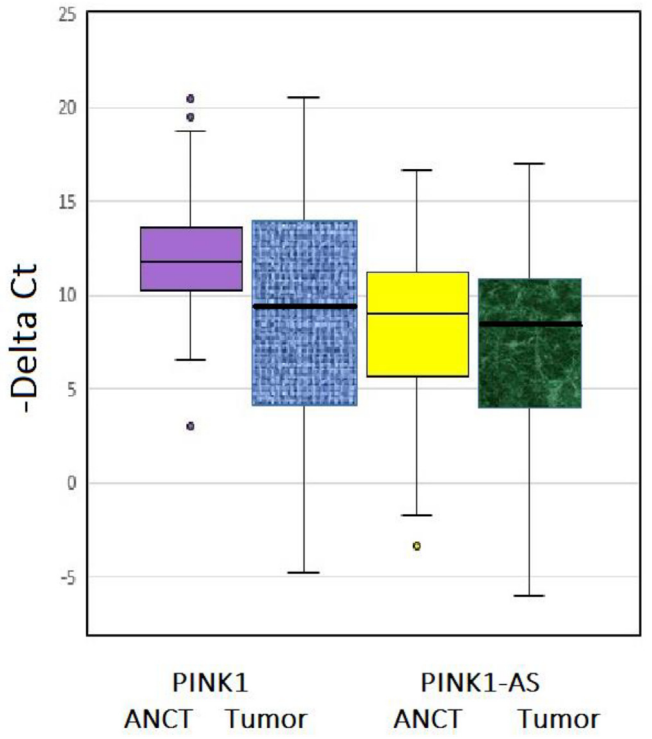

Fig. 1. Relative expression of *PINK1* and its NAT in malignant tissues and nearby tissues as designated by –delta CT amounts (CT $_{\text{reference gene}}$-CT $_{\text{target gene}}$).

CT $_{\text{target gene}}$).

3.3. Association between expression of genes and tumor aspects

We assessed relative expression of *PINK1* and its NAT in each malignant sample versus its paired nearby tissue and arranged patients to down−/−up-regulated clusters for each gene based on these figures. We successively gaged association between relative expression of these genes and tumor aspects (Table 3). Relative expression of *PINK1* was associated with mitotic rate (P = .03).

We also compared normalized expression of *PINK1* and *PINK1-AS* between clinicopathological-based subdivisions and found trends toward over-expression of *PINK1* in younger patient compared with older patients (P = .09) and in grade 2 tumors compared with grade 3 ones (P = .08) (Table 4).

3.4. Correlation between expression of PINK1 and its NAT in breast cancer samples and nearby tissues

We found substantial correlation between relative expressions of *PINK1* and its NAT in both malignant tissues and nearby tissues (Figs. 2 and 3). According to the R^2 values, the correlation was strong in tumoral tissues (R^2 = 0.89) and moderate in ANCTs (R^2 = 0.35).

3.5. ROC curve analysis

ROC curve analysis showed 79.6% specificity and 51.9% sensitivity for *PINK1* transcript levels in discrimination of breast cancer tissues from nearby tissues (Table 5 and Fig. 4).

4. Discussion

Several lines of evidences indicate similarities between cancer and neurodegeneration especially in the terms of signaling pathways that regulate cell survival and death and DNA damage repair (O'Flanagan et al., 2016). PINK1 is among molecules that is involved in the pathophysiology of both neurodegenerative conditions and malignancies (O'Flanagan et al., 2016). This protein is originally designated based on its stimulation by the tumor suppressor PTEN in malignant cells. Meanwhile, it is a controller of cell cycle transition whose silencing considerably decreased cell proliferation, colony formation and invasiveness in immortalized mouse embryonic fibroblasts (O'Flanagan et al., 2015). However, overexpression of the *PINK1* transcript did not suppress cell growth in some studied malignant cell lines (Unoki and Nakamura, 2001). On the other hand, PINK1 expression is both controlled by and controls PI3K/Akt signaling which support its role in the carcinogenesis process (O'Flanagan and O'Neill, 2014). The results of previous studies suggest that PINK1 might have different or opposite functions even in a certain type of malignancy as revealed by dual anti-apoptotic and growth-inhibitory functions in breast cancer cells (Berthier et al., 2011). According to the inconsistencies between former studies regarding the role of PINK1 in the development of cancer, we assessed its expression in breast tissue samples and demonstrated down-regulation of *PINK1* in invasive ductal carcinoma samples versus their nearby tissues. Such observed down-regulation of *PINK1* in tumoral tissues of breast is consistent with the proposed role for it in the BRCA1 related pathways based on its sequence homology with BRAP2 (Nakajima et al., 2003). So, we hypothesize that its down-regulation might mimic lack of BRCA1 expression in breast cancer cells. Future in vitro studies are necessary to evaluate this assumption.

We simultaneously assessed expression of its NAT namely *PINK1-AS* in the same cohort of patients and reported no difference in its expression between tumoral tissues and nearby tissues. *PINK1-AS* is a long non-coding RNA which stabilizes its sense coding RNA (Scheele et al., 2007). Such proposed role for *PINK1-AS* is supported by the observed positive correlation between expression levels of *PINK1* and *PINK1-AS* in both tumoral tissues and nearby tissues. The stronger pairwise correlation in tumoral tissues might reflect the occurrence of a novel regulatory route to compensate the decrease in the *PINK1* in tumoral tissues which needs to be judged in upcoming investigations.

We detected an association between *PINK1* expression and mitotic rate which is in line with the suggested function of this gene in the modulation of PI3K/Akt/mTOR signaling axis (O'Flanagan and O'Neill, 2014) and warrants functional studies to clarify the underlying mechanism and its practical application in cancer management. We also found trends toward over-expression of *PINK1* in younger patient compared with older patients (P = .09) and in grade 2 tumors versus grade 3 ones (P = .08). Supposing a tumor suppressor role for PINK1 in breast cancer (as suggested by the observed reduction of it in tumoral tissues compared with nearby tissues), higher expression of it in younger patients and in lower grades might reflect higher levels of PTEN expression in these subjects. Imminent investigations in larger sample sizes are required to verify these associations, reveal the underlying mechanism and assess its significance in patients' outcome.

As a final point, we measured the diagnostic power of *PINK1* expression levels in breast tissues and demonstrated that it can predict the presence of cancer with 79.6% specificity and 51.9% sensitivity. Based on its high specificity, it might be a putative biomarker in a panel of biomarkers for breast cancer.

Our study had some limitations. Although we only demonstrated down-regulation of *PINK1* in relation to the mitotic rate, a larger percentage of patients with down-regulation of this gene had a lower mitotic rate which is associated with higher survival. Therefore, it confuses the presentation of the *PINK1* as a contributor to breast cancer pathogenesis. Furthermore, *PINK1* showed no association with the different molecular subtypes or tumor grade which adds to a conflicting picture. Besides, although we hypothesized that down-regulation of this gene might mimic lack of BRAC1 expression, we did not assess this aspect in our data. We also did not assess other associated mechanistic PINK1 markers. Thus, the current results are preliminary results

Table 3
Association between relative expressions of genes in malignant tissues compared with nearby tissues and tumor features (For each parameter, there are different numbers of missing data).

	PINK1 up-regulation	*PINK1* down-regulation	*P* value	*PINK1-AS1* up-regulation	*PINK1-AS1* down-regulation	P value
Age			0.56			0.72
< 55 years	11 (32.4%)	23 (67.6%)		12 (35.3%)	22 (64.7%)	
≥55 years	5 (25%)	15 (75%)		8 (40%)	12 (60%)	
Stage			0.07			0.6
1	4 (25%)	12 (75%)		5 (31.3%)	11 (68.7%)	
2	3 (20%)	12 (80%)		4 (26.7%)	11 (73.3%)	
3	3 (18.8%)	13 (81.2%)		6 (37.5%)	10 (62.5%)	
4	4 (80%)	1(20%)		3 (60%)	2 (40%)	
Histological grade			0.1			0.92
1	2 (25%)	6 (75%)		2 (25%)	6 (75%)	
2	3 (13%)	20 (87%)		8 (34.8%)	15 (65.2%)	
3	7 (43.8%)	9 (56.3%)		6 (37.5%)	10 (62.5%)	
Mitotic rate			0.03			0.64
1	2 (10.5%)	17 (89.5%)		6 (31.6%)	13 (68.4%)	
2	6 (33.3%)	12 (66.7%)		7 (38.9%)	11 (61.1%)	
3	3 (60%)	2 (40%)		2 (40%)	3 (60%)	
Tumor size			0.4			0.58
< 2	4 (25%)	12 (75%)		6 (37.5%)	10 (62.5%)	
2–5	9 (27.3%)	24 (72.7%)		11 (33.3%)	22 (66.7%)	
> 5	1 (100%)	0 (0%)		1(100%)	0 (0%)	
ER status			0.33			0.65
Positive	11 (25.6%)	32 (74.4%)		15 (34.9%)	28 (65.1%)	
Negative	3 (50%)	3 (50%)		3 (50%)	3 (50%)	
PR status			0.11			0.46
Positive	8 (21.6%)	29 (78.4%)		11 (29.7%)	26 (70.3%)	
Negative	5 (45.5%)	6 (54.5%)		6 (54.5%)	5 (45.5%)	
Her2 status			0.71			0.6
Positive	4 (33.3%)	8 (66.7%)		5 (41.7%)	7 (58.3%)	
Negative	9 (25%)	27 (75%)		12 (33.3%)	24 (66.7%)	

Table 4
Association between expression quantities of genes in malignant tissues and clinical aspects (Mean (Standard deviation) values of Efficiency ^CT reference gene-Efficiency ^CT target gene are displayed).

	PINK1 expression	P value	*PINK1-AS1* expression	P value
Age				
< 55 years old vs. ≥55 years old	7.12 (2.56) vs. 6.21 (1.81)	0.09	3406.35 (86.84.56) vs. 7424.23 (21,455.68)	0.34
ER status				
ER(+) vs. ER(−)	4.19 (2.11) vs. 1.03 (2.52)	0.51	4166.08 (13,378.33) vs. 2086.09 (2719.47)	0.73
PR status				
PR(+) vs. PR(−)	3.66 (2.17) vs. 9.73 (2.19)	0.42	2632.01 (8284.69) vs. 1726.18 (2373.62)	0.73
HER2 status				
HER2 (+) vs. HER2 (−)	3.8 (1.29) vs. 5.47 (2.4)	0.82	830.67 (1894.92) vs. 3001.36 (8489.03)	0.38
Tumor grade				
Grade 1 vs. 2	2.05 (3.05) vs. 4.23 (1.25)	0.9	9503.13 (16,191.96) vs. 1128.86 (2255.95)	0.21
Grade 1 vs. 3	2.05 (3.05) vs. 1.49 (3.63)	0.25	9503.13 (16,191.96) vs. 5371.4 (17,653.05)	0. 71
Grade 2 vs. 3	4.23 (1.25) vs. 1.49 (3.63)	0.08	1128.86 (2255.95) vs. 5371.4 (17,653.05)	0.55

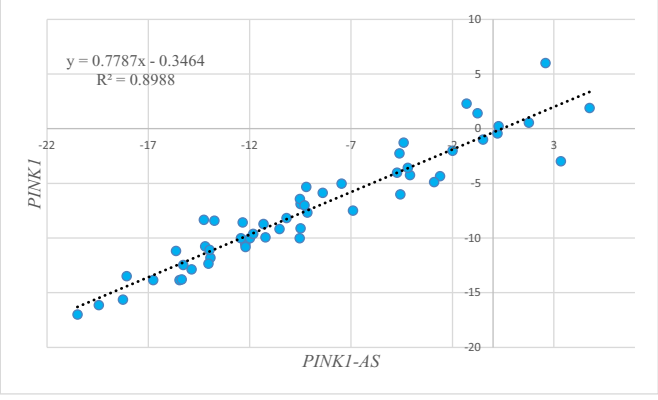

Fig. 2. Correlation between expression of *PINK1* and its NAT in breast cancer samples.

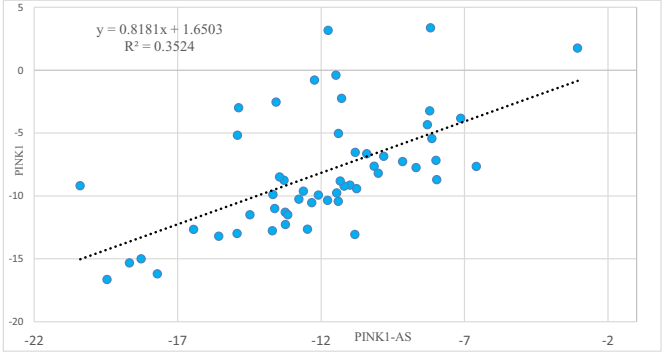

Fig. 3. Correlation between expression of *PINK1* and *PINK1-AS* in nearby tissues.

Table 5
Detailed information of ROC curve analysis (Estimate criterion: optimal cut-off point for gene expression).

	Estimate criterion	Area Under Curve	Youden index	Sensitivity	Specificity	*P*-value
PINK1 transcript levels	> −9.81	0.62	0.31	51.9	79.6	0.02

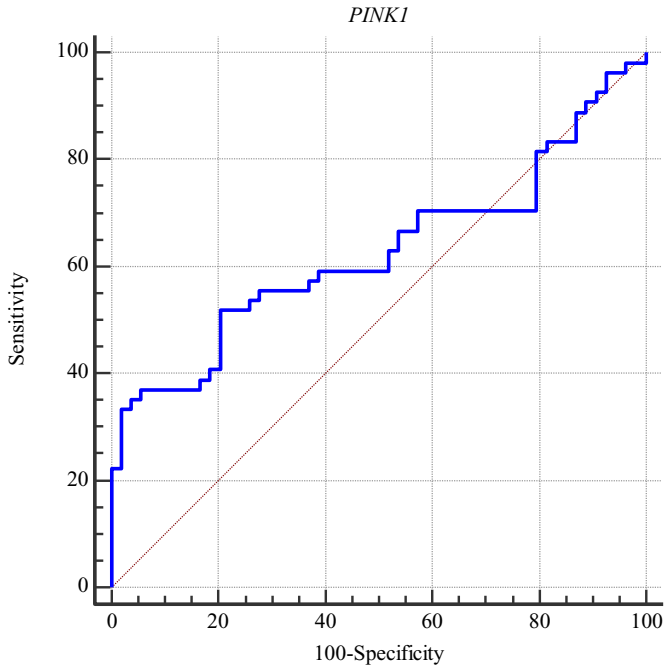

Fig. 4. The results of ROC curve analysis for assessment of the appropriateness of *PINK1* expression levels for discrimination of malignant tissues from nearby tissues.

needing mechanistical verifications.

Authors contribution

HY and HA performed the experiment. VKO analysed the data. MT and SGF designed and supervised the study. SGF wrote the draft and revised it. All authors contributed equally and fully aware of submission.

Declaration of Competing Interest

The authors declare they have no conflict of interest.

Acknowledgement

The present project was approved by a grant from Shahrekord University of Medical Sciences.

References

Bagchi, A., Mills, A.A., 2008. The quest for the 1p36 tumor suppressor. Cancer Res. 68, 2551–2556.
Berthier, A., Navarro, S., Jimenez-Sainz, J., Rogla, I., Ripoll, F., Cervera, J., Pulido, R., 2011. PINK1 displays tissue-specific subcellular location and regulates apoptosis and cell growth in breast cancer cells. Hum. Pathol. 42, 75–87.
Hammond, M.E.H., Hayes, D.F., Dowsett, M., Allred, D.C., Hagerty, K.L., Badve, S., Fitzgibbons, P.L., Francis, G., Goldstein, N.S., Hayes, M., 2010. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Archiv. Pathol. Lab. Med. 134, e48–e72.
Nakajima, A., Kataoka, K., Hong, M., Sakaguchi, M., Huh, N.H., 2003. BRPK, a novel protein kinase showing increased expression in mouse cancer cell lines with higher metastatic potential. Cancer Lett. 201, 195–201.
Nikpayam, E., Tasharrofi, B., Sarrafzadeh, S., Ghafouri-Fard, S., 2017. The role of long non-coding RNAs in ovarian cancer. Iran. Biomed. J. 21, 3–15.
O'Flanagan, C.H., O'Neill, C., 2014. PINK1 signalling in cancer biology. Biochim. Biophys. Acta 1846, 590–598.
O'Flanagan, C.H., Morais, V.A., Wurst, W., de Strooper, B., O'neill, C., 2015. The Parkinson's gene PINK1 regulates cell cycle progression and promotes cancer-associated phenotypes. Oncogene 34, 1363–1374.
O'Flanagan, C.H., Morais, V.A., O'neill, C., 2016. PINK1, cancer and neurodegeneration. Oncoscience 3, 1–2.
Scheele, C., Petrovic, N., Faghihi, M.A., Lassmann, T., Fredriksson, K., Rooyackers, O., Wahlestedt, C., Good, L., Timmons, J.A., 2007. The human PINK1 locus is regulated in vivo by a non-coding natural antisense RNA during modulation of mitochondrial function. BMC Genomics 8, 74.
Sinn, H.-P., Kreipe, H., 2013. A brief overview of the WHO classification of breast tumors. Breast Care 8, 149–154.
Unoki, M., Nakamura, Y., 2001. Growth-suppressive effects of BPOZ and EGR2, two genes involved in the PTEN signaling pathway. Oncogene 20, 4457–4465.
Valente, E.M., Bentivoglio, A.R., Dixon, P.H., Ferraris, A., Ialongo, T., Frontali, M., Albanese, A., Wood, N.W., 2001. Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am. J. Human Genet. 68, 895–900.
Wolff, A.C., Hammond, M., Schwartz, J.N., 2007. College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J. Clin. Oncol. 25, 118–145.