HHS Public Access

Author manuscript

Gynecol Oncol. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

Gynecol Oncol. 2016 December ; 143(3): 642–649. doi:10.1016/j.ygyno.2016.09.021.

Long non-coding RNAs, *ASAP1-IT1*, *FAM215A*, and *LINC00472*, in epithelial ovarian cancer

Yuanyuan Fu[a,b], **Nicoletta Biglia**[c], **Zhanwei Wang**[a], **Yi Shen**[a], **Harvey A. Risch**[d], **Lingeng Lu**[d], **Emilie Marion Canuto**[e], **Wei Jia**[a], **Dionyssios Katsaros**[e], and **Herbert Yu**[a,*]

[a]Cancer Epidemiology Program, University of Hawaii Cancer Center, United States

[b]Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, United States

[c]Department of Surgical Sciences, University of Turin, Torino, Italy

[d]Department of Chronic Disease Epidemiology, Yale School of Public Health, United States

[e]Department of Surgical Sciences, Azienda Ospedaliero-Universitaria, Turin, Italy

Abstract

Objective—Long non-coding RNAs (lncRNAs) are a class of non-protein coding transcripts that has gained significant attention lately due to their important biological actions and potential involvement in cancer. Ovarian cancer is a devastating disease with poor prognosis, and our understanding of lncRNA's involvement in the malignancy is limited. To further our knowledge, we measured the expression of three lncRNAs, *ASAP1-IT1, FAM215A*, and *LINC00472*, in tumor samples, and analyzed their associations with disease characteristics and patient survival.

*Corresponding author at: Cancer Epidemiology Program, University of Hawaii Cancer Center, 701 Ilalo Street, Suite 531, Honolulu, HI 96813, United States.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2016.09.021.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

E-Extra

Frozen tumor samples collected for study were evaluated by two independent pathologists to confirm that tumor cells were present in >80% of each tumor specimen. The tissue samples were pulverized using a tissue homogenizer. Samples of approximately 30 mg of pulverized tissue powder were used for total RNA extraction, which was performed using the AllPrep DNA/RNA Mini Kit (Qiagen). The extracted total RNAs were treated with RNase-free DNase to remove DNA contamination. The quality of the RNA samples was assessed by measuring light absorbance and RNA Integrity Number (RIN) using the NanoDrop spectrophotometer (NanoDrop 2000, Thermo Fisher) and Agilent 2100 Bioanalyzer System, respectively. The assessment showed a 260/280 ratio of 1.8 or higher and an average RIN number of 5.63 based on the 28s:18s rRNA ratio. High Capacity cDNA Reverse Transcription Kit was used to convert total RNA to cDNA (Applied Biosystems). The cDNA samples were analyzed for lncRNA and ASAP1 expression using the SYBR green-based real-time PCR (qPCR). The PCR reaction was performed in a LightCycler 480 instrument (Roche) using LightCycler 480 SYBR Green I Master with UDG (Roche). In the PCR reaction (10 μl), 1 μl cDNA template was mixed with 200 nM primers and 5 μl SYBR PCR master mix (LifeTech). The PCR reaction conditions included incubation at 50 °C for 2 min to activate UDG, 95 °C for 2 min to activate Taq polymerase, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Melting curves were generated after each PCR run to evaluate the size of PCR products. Each sample was tested in triplicate, and the mean value of three reactions was used for analysis if the coefficient of variation was <10%. If not, the mean of two closest reactions was used. As an internal reference, *GAPDH* expression was also measured simultaneously with lncRNAs and *ASAP1* in all of the tumor samples. Primer sequences for the PCR reactions are provided in Supplementary Table 1.

E-component

The following are the supplementary data related to this article.

Methods—Two hundred sixty-six patients diagnosed with primary epithelial ovarian cancers were recruited for the study. Fresh-frozen tumor samples were obtained from the patients at tumor resection and analyzed by RT-qPCR for expression of *ASAP1-IT1, FAM215A*, and *LINC00472*. Associations of lncRNA expression with patient survival were determined using Cox proportional hazards regression models.

Results—We observed high expression of *ASAP1-IT1, FAM215A* and *LINC00472* more frequently in low grade tumors and early stage disease compared to high grade tumors and late stage disease, respectively. High expression of *ASAP1-IT1* and *FAM215A* were associated with favorable overall survival, and the survival association with *ASAP1-IT1* was independent of tumor grade and disease stage. Analyses of online data also demonstrated similar survival associations with *ASAP1-IT1* and *FAM215A*, suggesting that these lncRNAs may be involved in ovarian cancer progression.

Conclusions—LncRNAs may play appreciable roles in ovarian cancer and more research is needed to elucidate their biological mechanisms and clinical implications in tumor characterization as well as disease prognosis and treatment.

Keywords

ASAP1-IT1; FAM215A; LNC00472; ASAP1; Ovarian cancer; Prognosis

1 Introduction

Ovarian cancer is the most lethal gynecological malignancy, attributable to 5% of female cancer deaths in the US [1]. >50% of ovarian cancer patients succumb to the tumor within 5 years of diagnosis. It is believed that ovarian cancer survival may be significantly improved if the disease can be detected early when the tumors are still confined to the ovaries [1]. Since only a small percentage of patients are diagnosed with localized disease, a means that allows detection of ovarian cancer at early stages is urgently needed. Further elucidating the molecular features of ovarian cancer may help to achieve this goal. Previous knowledge of ovarian cancer biology has been largely centered on proteins and their coding genes. Since only 2% of the human genome encodes proteins [2], our understanding of ovarian cancer from the genome perspective is quite limited. It is now known that >90% of the genome is transcribed into RNAs, and a majority of them are non-coding RNAs. Many of these non-coding RNAs are biologically functional, and are involved in regulation of cell activities and functions. Dysregulation of non-coding RNAs may play an important role in various pathogenic processes of human diseases including cancer [3–5].

Non-coding RNAs with sequences of 200 nucleotides or more are called long non-coding RNAs (lncRNAs) [6]. In ovarian cancer initiation and progression, little is known about the role of lncRNAs. In this report, we studied the expression of three lncRNAs, *ASAP1-IT1, FAM215A* and *LINC00472*, in primary epithelial ovarian cancer, and analyzed their relationships with tumor characteristics and disease outcomes. These lncRNAs were selected for study either because our previous investigations provided evidence of its potential involvement in cancer (*LINC00472*) or our analyses using the Kaplan-Meier Plotter (http://kmplot.com/analysis/) of online databases suggested their possible associations with ovarian

cancer survival (*ASAP1-IT1, FAM215A*) [7]. *LINC00472* is a long intergenic non-coding RNA located on chromosome 6q13. Our recent studies revealed that this lincRNA may be associated with tumor suppression in breast cancer [8, 9]. *ASAP1-IT1* is an intronic transcript of the *ASAP1* (*AMAP1*; *DDEF1*) gene which encodes ASAP1, an ADP-ribosylation factor (ARF) GTPase-activating protein involved in membrane trafficking and cytoskeleton remodeling [10, 11]. Reports have suggested that *ASAP1* is associated with tumor metastasis and poor cancer survival [12–14]. LncRNA *ASAP1-IT1* may antagonize the function of *ASAP1*, and our analysis of online data showed that high expression of *ASAP1-IT1* was associated with favorable survival in ovarian cancer. A similar association was also observed in the public database for another lncRNA, *FAM215A* (family with sequence similarity 215 member A, or *C17orf88, LINC00530*). Currently, little is known about *FAM215A* with regard to its biologic activities and associations with cancer outcomes.

2 Materials and methods

2.1 Patient information

Patients with epithelial ovarian cancer were recruited from two hospitals affiliated with the University of Turin in Turin, Italy. Patient enrollment occurred between October 1991 and February 2000 in one hospital (group one: n = 191), and between April 1997 and January 2013 in the other (group two: n = 75). All patients enrolled in the study underwent cytoreduction surgery for primary ovarian cancer, and 208 (78%) of these patients received standard post-operative platinum-based chemotherapy after surgery, which included cisplatin and cyclophosphamide between 1991 and 1995 (n = 63) and carboplatin and paclitaxel after 1995 (n = 145). Fresh tumor samples were collected from the patients during surgery. The specimens were snap-frozen in liquid nitrogen immediately after resection and then transferred to − 80°C freezers for storage. Patient information on age at surgery, disease stage, tumor grade and histology was obtained from medical records and pathology reports. Disease stage and tumor grade were categorized based on the International Federation of Gynecology and Obstetrics (FIGO) Classification and the WHO Guidelines, respectively [15, 16]. Patients were followed for disease progression and survival outcomes from surgery through June 2005 (group one: n = 180) or through March 2015 (group two: n = 68). The median follow-up time was 29.9 months (range: 0.6–114.1) for the former group and 40.5 months (range: 3.4–165.5) for the latter, respectively. Information on treatment response was available for 179 patients, including 128 who had complete response and 51 who did not. Treatment response was assessed one month after chemotherapy, and complete response was defined as resolution of all evidence of the disease for at least a month. The study was approved by ethics review committees at the hospitals, and informed consent was obtained from each patient who participated in the study.

2.2 Tumor analysis

Frozen tumor samples collected for study were evaluated by two independent pathologists to confirm that tumor cells were present in > 80% of each tumor specimen. The tissue samples were pulverized using a tissue homogenizer. Samples of approximately 30 mg of pulverized tissue powder were used for total RNA extraction, which was performed using the AllPrep DNA/RNA Mini Kit (Qiagen). The extracted total RNAs were treated with RNase-free

DNase to remove DNA contamination. The quality of the RNA samples was assessed by measuring light absorbance and RNA Integrity Number (RIN) using the NanoDrop spectrophotometer (NanoDrop 2000, Thermo Fisher) and Agilent 2100 Bioanalyzer System, respectively. The assessment showed a 260/280 ratio of 1.8 or higher and an average RIN number of 5.63 based on the 28s:18s rRNA ratio. High Capacity cDNA Reverse Transcription Kit was used to convert total RNA to cDNA (Applied Biosystems). The cDNA samples were analyzed for lncRNA and *ASAP1* expression using the SYBR green-based real-time PCR (qPCR). The PCR reaction was performed in a LightCycler 480 instrument (Roche) using LightCycler 480 SYBR Green I Master with UDG (Roche). In the PCR reaction (10 μl), 1 μl cDNA template was mixed with 200 nM primers and 5 μl SYBR PCR master mix (LifeTech). The PCR reaction conditions included incubation at 50 °C for 2 min to activate UDG, 95 °C for 2 min to activate Taq polymerase, and 40 cycles of 95 °C for 15s and 60°C for 1 min. Melting curves were generated after each PCR run to evaluate the size of PCR products. Each sample was tested in triplicate, and the mean value of three reactions was used for analysis if the coefficient of variation was <10%. If not, the mean of two closest reactions was used. As an internal reference, *GAPDH* expression was also measured simultaneously with lncRNAs and *ASAP1* in all of the tumor samples. Primer sequences for the PCR reactions are provided in Supplementary Table 1.

2.3 Statistical analysis

Expression index (EI) was calculated as levels of RNA expression for *ASAP1-IT1, FAM215A*, and *LINC00472* after adjusting for *GAPDH*, and calculation was based on the formula $1000 \times 2^{(-\Delta Ct)}$, where Ct is the cycle threshold and Δ Ct is the difference between Ct_{lncRNA} and Ct_{GAPDH}. After calculation, EI values were grouped into 3 categories, low, medium and high, based on the tertile distribution of each lncRNA among the patients. The ordinal values were then analyzed for their associations with clinical and pathological variables, using the Chi-square test or Mann-Whitney U statistics where appropriate. Survival analyses were performed using the Cox proportional hazards regression model at both univariate and multivariate levels. In the multivariate analyses, age at surgery, disease stage, tumor grade and histological type were included for adjustment. The log-rank test with two degree of freedom was used for comparison of three Kaplan-Meier survival curves. Two survival endpoints, progression-free survival and overall survival, were studied as disease outcomes. Progression-free survival was the time interval from the date of surgery to the date of disease progression or last follow-up; overall survival was the time between surgery and last follow-up or death. SAS (version 9.4) and R (version 3.0.2) were used for statistical analyses. All p-values were two-sided.

3 Results

In total, 266 patients were included in the study. The median age of patients at surgery was 59.1 years, ranging from 24.4 to 82.1 years. Of the patients, 73 (28%) had stage I or II disease, and 191 (72%) had stage III or IV disease. Twenty-nine patients (11%) had grade 1 tumors, and 235 (89%) had grade 2 or 3 tumors. Two patients had no information either on tumor grade or disease stage. Forty-five percent of the patients (n = 121) were diagnosed with serous tumors, and 55% (n = 145) had other histotypes, including endometrioid,

mucinous, clear cell and undifferentiated. Median levels of lncRNA expression were 3.57 EI (range: 0.03–83.33) for *ASAP1-IT1*, 0.62 EI (range: 0.02–23.85) for *FAM215A*, and 8.44 EI (range: 0.03–289.84) for *LINC00472*. The ranges of each tertile group were: 0.03–2.36 (low), 2.36–5.43 (mid) and 5.43–83.33(high) for *ASAP1-IT1*; 0.018–0.39 (low), 0.39–0.92 (mid) and 0.92–23.85 (high) for *FAM215A*; and 0.03–5.5 (low), 5.5–13.67 (mid) and 13.67–289.84 (high) for *LINC00472*. Table 1 shows the associations of lncRNA expression with clinical and pathological variables. No correlations were observed between lncRNA expression and patient age at surgery, except for *FAM215A*. Disease stage and tumor grade were associated with expression of *LINC00472* and *FAM215A*. High expression of these lncRNAs correlated with lower tumor grades and earlier disease stages. For *FAM215A*, 45.21% of patients with stage I or II disease had high expression compared to 27.89% of those with stage III or IV patients ($p = 0.0072$), and 51.72% of patients with grade 1 tumors had high expression compared to 30.34% of patients with grade 2 or 3 tumors ($p = 0.004$). Patients with high expression of FAM215A were more likely to respond to chemotherapy compared to those with low expression ($p = 0.017$). For *LINC00472*, the corresponding numbers were 45.21% *versus* 28.04% when comparing disease stage ($p = 0.024$), and 51.72% *versus* 30.47% when comparing tumor grade ($p = 0.004$). *ASAP1-IT1* expression appeared to differ by disease stage ($p = 0.025$), but no significant trend was observed with regard to the difference by tumor grade. Expression of *LINC00472, FAM215A* and *ASAP1-IT1* did not show appreciable differences between serous and non-serous tumors.

The results of survival analyses are shown in Table 2. *ASAP1-IT1* expression was associated with overall survival, but not with progression-free survival (Fig. 1A and B). Patients with high expression had better overall survival compared to those with low expression, and the survival association remained significant after adjustment for patient age, disease stage, tumor grade and histologic type. *FAM215A* expression was associated with overall survival and possibly with progression-free survival, but these associations were observed only in univariate analysis (Fig. 1C and D). After adjusting for disease stage, tumor grade and histology, the survival associations with *FAM215A* were no longer significant. *LINC00472* expression was not associated with either progression-free or overall survival (Fig. 1E and F). Since tumor grade and disease stage were associated with some of the lncRNA expression, we also performed survival analyses among patients stratified by their tumor grade or disease stage. These additional analyses did not change the study results substantially (data not shown).

ASAP1-IT1 is transcribed from an intron of the *ASAP1* gene, and may regulate the activity and function of ASAP1. We measured the mRNA expression of *ASAP1* in ovarian cancer and analyzed its association with the disease characteristics and *ASAP1-IT1* expression. *ASAP1* expression appeared to be higher in high grade than in low grade tumors ($p = 0.048$) (Table 1), and high expression was associated with increased risk of tumor progression, although no association was observed for overall survival (Table 2, Fig. 2A and B). Expression of *ASAP1-IT1* and *ASAP1* were positively correlated in ovarian tumors (Spearman $r = 0.645$; $p < 0.001$).

Using the Kaplan-Meier Plotter (http://kmplot.com/analysis/), we found that high expression of *ASAP1-IT1* was also associated with favorable overall survival of ovarian cancer patients

(p = 0.0047) (Fig. 3A), but no association with progression-free survival (Fig. 3B). Similarly, analyses of online data showed that high expression of *FAM215A* was significantly associated with favorable overall survival (p = 0.00080) (Fig. 3C) and progression-free survival (p = 0.0059) (Fig. 3D). *LINC00472* was not associated with either overall survival (Fig. 3E) or progression-free survival (Fig. 3F). We also checked the association of *ASAP1* with patient survival using the online data, and found high expression associated with poor progression-free survival, but not with overall survival (Fig. 4A and B).

4 Discussion

Our study showed that tumor expression of two lncRNAs, *LINC00472* and *FAM215A*, differed significantly by tumor grade and disease stage: high expression associated with low tumor grades and early disease stages. *ASAP1-IT1* had a similar trend in association with disease stage, but not tumor grade. High expression of *ASAP1-IT1* and *FAM215A* were also associated with more favorable overall survival of ovarian cancer. Similar survival associations were further observed in an online dataset [7], indicating that the findings were somehow consistent across different studies. We also analyzed the expression of *ASAP1* and found high expression associated with poor progression-free survival. Furthermore, *ASAP1* expression was positively correlated with *ASAP1-IT1* expression, suggesting that the effects of *ASAP1-IT1* on ASAP1, if any, may not be achieved through its down-regulation of *ASAP1* expression.

Based on their locations in or relative to the coding genes, lncRNAs are classified into intronic, intergenic, and overlapping (either in sense or antisense orientation) transcripts [17]. Several studies have reported that some intronic non-coding RNAs are positively correlated with expression of their corresponding protein-coding genes, whereas others are inversely correlated [18–21]. These observations indicate potentially complex regulations between intronic lncRNAs and their surrounding genes. Non-coding RNAs have been found to act on their targets either at the transcriptional level or post-transcriptionally. The mechanisms that determine the pre- and post-transcription regulation remain to be elucidated. *ASAP1-IT1* is located in an intron of the *ASAP1* gene, and the biological functions of *ASAP1-IT1* are still unknown despite the fact that the *ASAP1* gene has been well characterized. Evidence suggests that *ASAP1* may be an oncogene as *ASAP1* expression is highly up-regulated in a variety of tumors in comparison with normal tissue, and in colorectal cancer, the expression correlates with poor prognosis. *ASAP1* enhances metastasis *in vivo*, and stimulates tumor cell migration, invasion, and adhesion *in vitro* [12]. Studies have indicated that *ASAP1* is highly expressed in primary prostate cancer and metastatic prostate tumors compared to benign prostate tissue. Down-regulation of *ASAP1* in PC-3 markedly inhibits cell migration and invasion [14]. *ASAP1* also enhances the invasion of breast cancer cells [22]. Recently, Hou et al. found that *ASAP1* expression was higher in epithelial ovarian cancer than in normal ovarian tissue, and high expression was associated with poor progression-free and overall survivals [13]. In our study, we found a similar association between *ASAP1* expression and progression-free survival, though not with overall survival. We also found that *ASAP1* expression was positively associated with tumor grade. How *ASAP1* may be regulated by lncRNA *ASAP1-IT1* remains unknown. The finding in our study suggests that *ASAP1-IT1* may not antagonize the action of *ASAP1*

through suppressing its expression. Previous studies have found positive correlations between the expression of host genes and their associated intronic lncRNAs [19, 20]. Sometimes, lncRNAs are not simply co-expressed with their host genes, and their expression may be independent of the host genes. Intronic lncRNAs can modulate the biological pathways of their host genes. For example, *SPRY4* is an inhibitor of the receptor-transduced mitogen-activated protein kinase (MAPK) signaling pathway. *SPRY4-IT1*, which is transcribed from an intron of the *SPRY4* gene, can affect the MAPK signaling pathway through its interaction with Raf1, B-Raf, MEK1/2, TESK1, MARKK, and MARK2 [23]. Intronic lncRNAs can also negatively regulate their host genes. *NPTN* is overexpressed in breast cancer cells resulting in significant tumor growth, but its intronic lncRNA, *lnc-LET*, is a tumor suppressor that appears to inhibit the metastasis of hepatocellular carcinoma [5].

Currently, few studies have evaluated the function of *FAM215A*. One report showed that suppression of *FAM215A* expression by siRNA increased the death of A375 melanoma cells induced by MLN4924 [24]. Although our study and the data from online sources indicated a possible association with ovarian cancer survival, the biological relevance of *FAM215A* in ovarian cancer is still unclear.

LINC00472 has been observed by our group to be involved in breast cancer. In our previous studies, we found that high expression of *LINC00472* was associated with favorable overall survival of breast cancer patients in our study as well as in >2 dozen clinical data sets available online [8, 9]. This finding was consistent across study populations and with different analytical technologies, as well as supported by *in vitro* experiments. Using breast cancer cell lines to manipulate the expression of *LINC00472*, we demonstrated that increasing *LINC00472* expression was associated with reduced cell proliferation and migration. Since ovarian cancer shares certain aspects in etiology with breast cancer as suggested by *BRCA1* and *BRCA2* mutations, we analyzed *LINC00472* expression in epithelial ovarian cancer, the most common form, and its associations with disease characteristics and patient survival [25]. In our analysis, although we did not find any associations between the lncRNA expression and disease outcome, we did observe that high expression of *LINC00472* was associated with low grade tumors and early stage disease. These findings are consistent with what we have seen in breast cancer, offering more evidence in support of the speculation that *LINC00472* may play a role in cancer as a tumor suppressor.

In summary, we investigated the clinical significance of three lncRNAs, *LINC00472, ASAP1-IT1* and *FAM215A*, in ovarian cancer, and found that two of the lncRNAs, *LINC00472* and *FAM215A*, were associated with tumor grade and disease stage. Furthermore, expression of *FAM215A* and *ASAP1-IT1* were associated with disease outcomes, and these associations were also seen in other datasets. Our findings suggest that lncRNAs may play appreciable roles in cancer and that more research is needed to elucidate the biological mechanisms of lncRNAs involved in tumorigenesis and disease progression, as well as their clinical implications in tumor characterization and patient management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Cancer Facts & Figures 2015. American Cancer Society; Atlanta: 2015.
2. Kapranov P, Willingham AT, Gingeras TR. Genome-wide transcription and the implications for genomic organization. Nat. Rev. Genet. 2007; 8:413–423. [PubMed: 17486121]
3. Li L, Liu B, Wapinski OL, Tsai MC, Qu K, Zhang J, et al. Targeted disruption of Hotair leads to homeotic transformation and gene derepression. Cell Rep. 2013; 5:3–12. [PubMed: 24075995]
4. Sleutels F, Zwart R, Barlow DP. The non-coding Air RNA is required for silencing autosomal imprinted genes. Nature. 2002; 415:810–813. [PubMed: 11845212]
5. Zhang B, Arun G, Mao YS, Lazar Z, Hung G, Bhattacharjee G, et al. The lncRNA Malat1 is dispensable for mouse development but its transcription plays a cis-regulatory role in the adult. Cell Rep. 2012; 2:111–123. [PubMed: 22840402]
6. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol. Cell. 2011; 43:904–914. [PubMed: 21925379]
7. Gyorffy B, Lanczky A, Szallasi Z. Implementing an online tool for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients. Endocr. Relat. Cancer. 2012; 19:197–208. [PubMed: 22277193]
8. Shen Y, Katsaros D, Loo LW, Hernandez BY, Chong C, Canuto EM, et al. Prognostic and predictive values of long non-coding RNA LINC00472 in breast cancer. Oncotarget. 2015; 6:8579–8592. [PubMed: 25865225]
9. Shen Y, Wang Z, Loo LW, Ni Y, Jia W, Fei P, et al. LINC00472 expression is regulated by promoter methylation and associated with disease-free survival in patients with grade 2 breast cancer. Breast Cancer Res. Treat. 2015; 154:473–482. [PubMed: 26564482]
10. D'Souza-Schorey C, Chavrier P. ARF proteins: roles in membrane traffic and beyond. Nat. Rev. Mol. Cell Biol. 2006; 7:347–358. [PubMed: 16633337]
11. Gillingham AK, Munro S. The small G proteins of the Arf family and their regulators. Annu. Rev. Cell Dev. Biol. 2007; 23:579–611. [PubMed: 17506703]
12. Muller T, Stein U, Poletti A, Garzia L, Rothley M, Plaumann D, et al. ASAP1 promotes tumor cell motility and invasiveness, stimulates metastasis formation in vivo, and correlates with poor survival in colorectal cancer patients. Oncogene. 2010; 29:2393–2403. [PubMed: 20154719]
13. Hou T, Yang C, Tong C, Zhang H, Xiao J, Li J. Overexpression of ASAP1 is associated with poor prognosis in epithelial ovarian cancer. Int. J. Clin. Exp. Pathol. 2014; 7:280–287. [PubMed: 24427349]
14. Lin D, Watahiki A, Bayani J, Zhang F, Liu L, Ling V, et al. ASAP1, a gene at 8q24, is associated with prostate cancer metastasis. Cancer Res. 2008; 68:4352–4359. [PubMed: 18519696]
15. Shepherd JH. Revised FIGO staging for gynaecological cancer. Br. J. Obstet. Gynaecol. 1989; 96:889–892. [PubMed: 2775686]
16. Scully RE, Sobin LH. Histologic typing of ovarian tumors. Arch. Pathol. Lab. Med. 1987; 111:794–795. [PubMed: 3632298]
17. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu. Rev. Biochem. 2012; 81:145–166. [PubMed: 22663078]
18. Nakaya HI, Amaral PP, Louro R, Lopes A, Fachel AA, Moreira YB, et al. Genome mapping and expression analyses of human intronic noncoding RNAs reveal tissue-specific patterns and enrichment in genes related to regulation of transcription. Genome Biol. 2007; 8:R43. [PubMed: 17386095]
19. Mercer TR, Dinger ME, Sunkin SM, Mehler MF, Mattick JS. Specific expression of long noncoding RNAs in the mouse brain. Proc. Natl. Acad. Sci. U. S. A. 2008; 105:716–721. [PubMed: 18184812]

20. Dinger ME, Amaral PP, Mercer TR, Pang KC, Bruce SJ, Gardiner BB, et al. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome Res. 2008; 18:1433–1445. [PubMed: 18562676]

21. Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, et al. Antisense transcription in the mammalian transcriptome. Science. 2005; 309:1564–1566. [PubMed: 16141073]

22. Onodera Y, Hashimoto S, Hashimoto A, Morishige M, Mazaki Y, Yamada A, et al. Expression of AMAP1, an ArfGAP, provides novel targets to inhibit breast cancer invasive activities. EMBO J. 2005; 24:963–973. [PubMed: 15719014]

23. Khaitan D, Dinger ME, Mazar J, Crawford J, Smith MA, Mattick JS, et al. The melanoma-upregulated long noncoding RNA SPRY4-IT1 modulates apoptosis and invasion. Cancer Res. 2011; 71:3852–3862. [PubMed: 21558391]

24. Blank JL, Liu XJ, Cosmopoulos K, Bouck DC, Garcia K, Bernard H, et al. Novel DNA damage checkpoints mediating cell death induced by the NEDD8-activating enzyme inhibitor MLN4924. Cancer Res. 2013; 73:225–234. [PubMed: 23100467]

25. King MC, Marks JH, Mandell JB. New York Breast Cancer Study G. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003; 302:643–646. [PubMed: 14576434]

Highlights

- *ASAP1-IT1, FAM215A* and *LINC00472* were highly expressed in early stage disease of EOC.
- *ASAP1-IT1, FAM215A* and *LINC00472* were also highly expressed in low grade tumors.
- High expression of *ASAP1-IT1* and *FAM215A* was associated with favorable OS.
- Large online database showed similar survival associations with *ASAP1-IT1* and *FAM215A*.

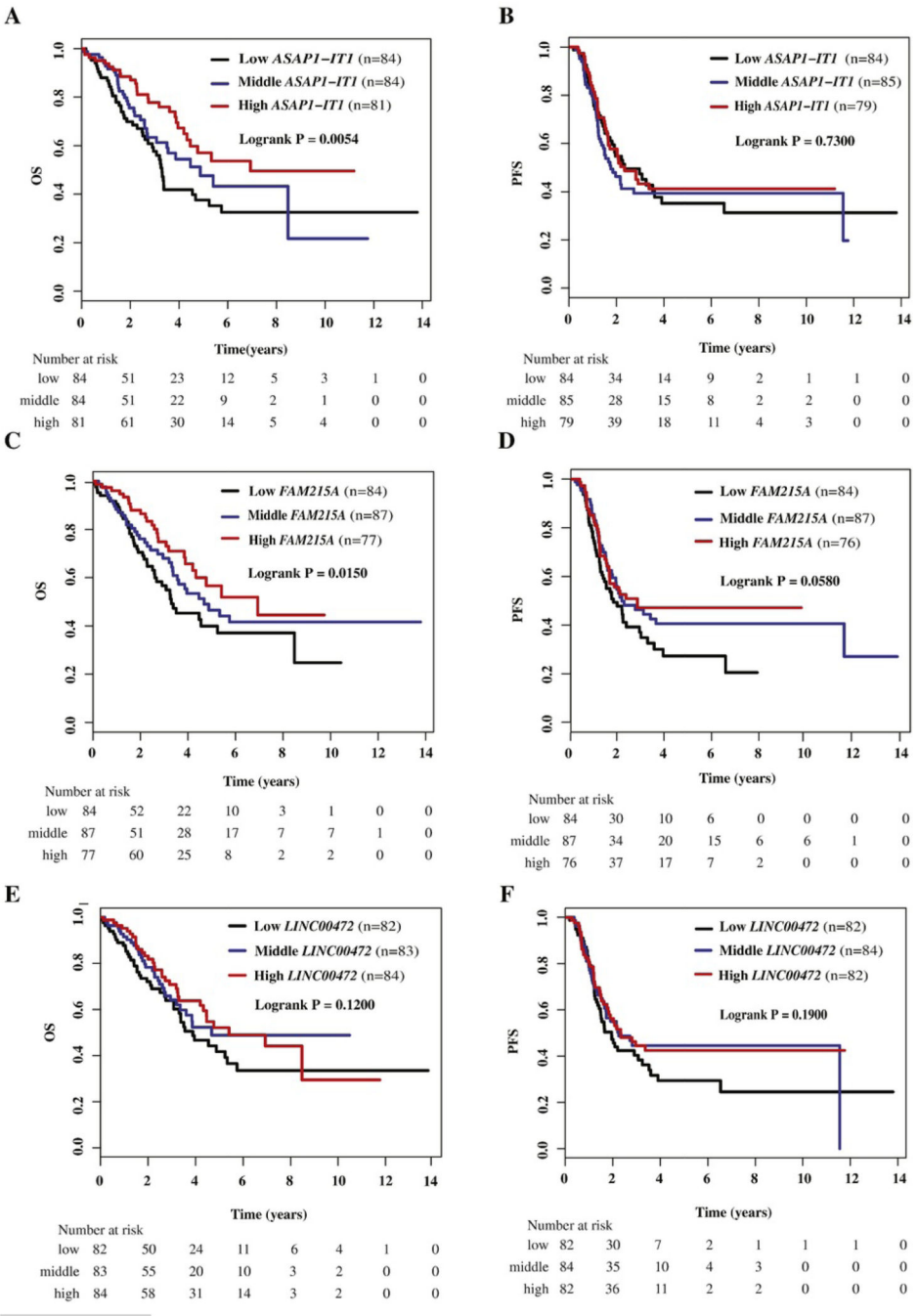

Fig. 1.

Kaplan-Meier survival by tertiles of *ASAP1-IT1, FAM215A* and *LINC00472* expression in ovarian cancer patients. A) Overall survival (OS) by low, middle, and high *ASAP1-IT1*; B) progression-free survival (PFS) by low, middle, and high *ASAP1-IT1*; C) overall survival (OS) by low, middle, and high *FAM215A*; D) progression-free survival (PFS) by low, middle, and high *FAM215A*; E) overall survival (OS) by low, middle, and high *LINC00472*; F) progression-free survival (PFS) by low, middle, and high *LINC00472*.

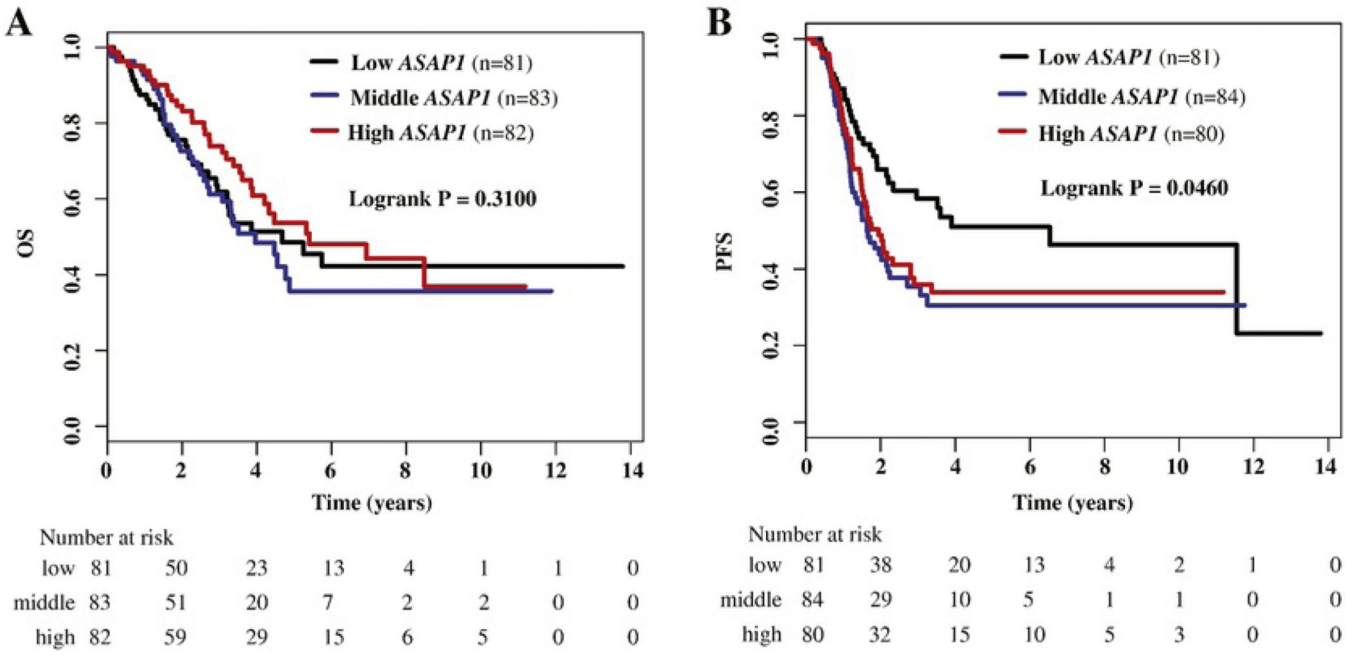

Fig. 2.

Kaplan-Meier survival by tertiles of *ASAP1* expression in ovarian cancer patients. A) Overall survival (OS) by low, middle, and high *ASAP1*; B) progression-free survival (PFS) by low, middle, and high *ASAP1*.

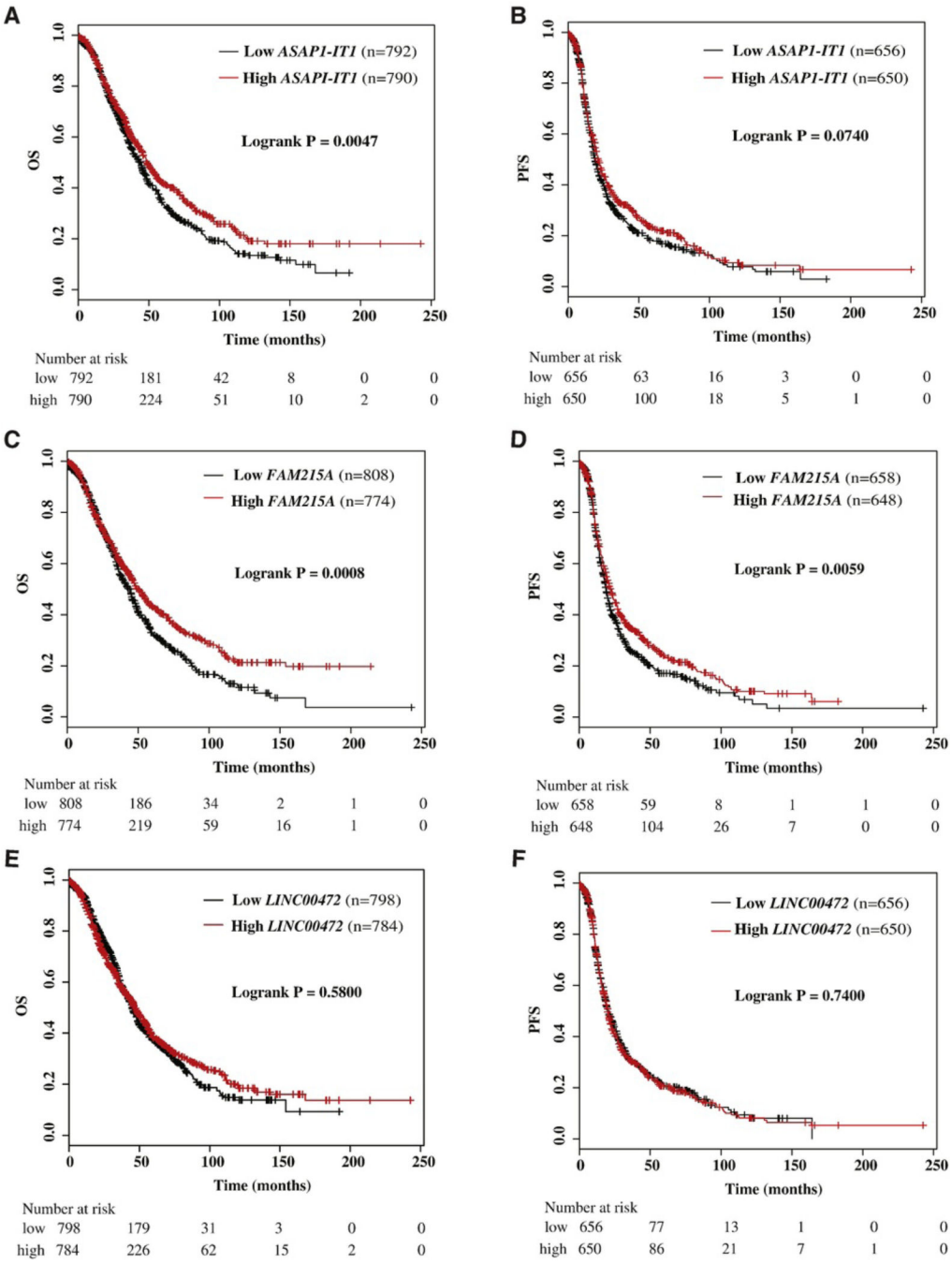

Fig. 3.

Kaplan-Meier survival by expression tertiles of *ASAP1-IT1, FAM215A* and *LINC00472* in an online database analyzed by Kaplan-Meier Plotter. A) Overall survival (OS) by low and high *ASAP1-IT1*; B) progression-free survival (PFS) by low and high *ASAP1-IT1*; C) overall survival (OS) by low and high *FAM215A*; D) progression-free survival (PFS) by low and high FAM215A; E) overall survival (OS) by low and high *LINC00472*; F) progression-free survival (PFS) by low and high *LINC00472*.

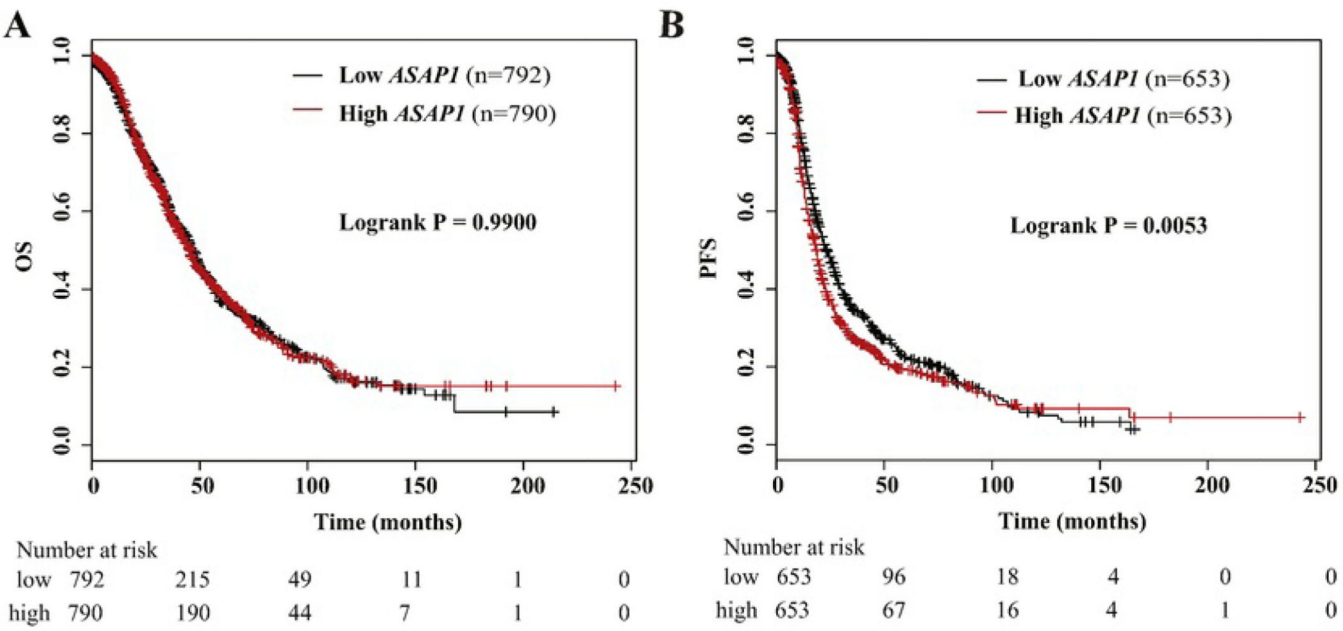

Fig. 4.

Kaplan-Meier survival by expression tertiles of *ASAP1* in an online database analyzed by Kaplan-Meier Plotter. A) Overall survival (OS) by low and high *ASAP1*; B) progression-free survival (PFS) by low and high *ASAP1*.

Table 1

Associations of lncRNAs and *ASAP1* with clinical and pathological variables.

Variables	Total no. (%)	Low no. (%)	Mid no. (%)	High no. (%)	p value
ASAP1-IT1					
Age					0.19
≥ 59.08	121 (46.18)	41 (33.88)	35 (28.93)	45 (37.19)	
< 59.08	141 (53.82)	45 (31.91)	55 (39.01)	41 (29.08)	
Disease stage					**0.025**
I–II	73 (27.65)	22 (30.14)	18 (24.66)	33 (45.21)	
III–IV	191 (72.35)	66 (34.55)	71 (37.17)	54 (28.27)	
Tumor grade					0.55
1	29 (10.98)	8 (27.59)	9 (31.03)	12 (41.38)	
2–3	235 (89.02)	80 (34.04)	81 (34.47)	74 (31.49)	
Histologic type					0.79
Non-serous	145 (54.51)	49 (33.79)	47 (32.41)	49 (33.79)	
Serous	121 (45.49)	39 (32.23)	44 (36.36)	38 (31.40)	
Treatment response[a]					0.59
Yes	128 (71.51)	40 (31.25)	42 (32.81)	46 (35.94)	
No	51 (28.49)	20 (39.22)	15 (29.41)	16 (31.37)	
FAM215A					
Age					**0.028**
≥ 59.08	120 (45.98)	46 (38.33)	31 (25.83)	43 (35.83)	
< 59.08	141 (54.02)	39 (27.66)	58 (41.13)	44 (31.21)	
Disease stage					**0.0072**
I–II	73 (27.76)	15 (20.55)	25 (34.25)	33 (45.21)	
III–IV	190 (72.24)	73 (38.42)	64 (33.68)	53 (27.89)	
Tumor grade					**0.004**
1	29 (11.03)	2 (6.90)	12 (41.38)	15 (51.72)	
2–3	234 (88.97)	86 (36.75)	77 (32.91)	71 (30.34)	

Gynecol Oncol. Author manuscript; available in PMC 2017 December 01.

Variables	Total no. (%)	Low no. (%)	Mid no. (%)	High no. (%)	p value
Histologic type					0.85
Non-serous	144 (54.34)	47 (32.64)	47 (32.64)	50 (34.72)	
Serous	121 (45.66)	41 (33.88)	42 (34.71)	38 (31.40)	
Treatment response[a]					**0.017**
Yes	128 (71.91)	38 (29.69)	41 (32.03)	49 (38.28)	
No	50 (28.09)	20 (40.00)	22 (44.00)	8 (16.00)	
LINC00472					
Age					0.65
≥ 59.08	121 (46.54)	41 (33.88)	37 (30.58)	43 (35.54)	
< 59.08	139 (53.46)	44 (31.65)	50 (35.97)	45 (32.37)	
Disease stage					**0.024**
I–II	73 (27.86)	18 (24.66)	22 (30.14)	33 (45.21)	
III–IV	189 (72.14)	70 (37.04)	66 (34.92)	53 (28.04)	
Tumor grade					**0.004**
1	29 (11.07)	2 (6.90)	12 (41.38)	15 (51.72)	
2–3	233 (88.93)	86 (36.91)	76 (32.62)	71 (30.47)	
Histologic type					0.21
Non-serous	145 (54.92)	55 (37.93)	46 (31.72)	44 (30.34)	
Serous	119 (45.08)	33 (27.73)	42 (35.29)	44 (36.97)	
Treatment response[a]					0.096
Yes	128 (71.51)	40 (31.25)	39 (30.47)	49 (38.28)	
No	51 (28.49)	19 (37.25)	21 (41.18)	11 (21.57)	
ASAP1					
Age					0.40
≥ 59.08	119 (46.12)	40 (33.61)	36 (30.25)	43 (36.13)	
< 59.08	139 (53.88)	43 (30.94)	53 (38.13)	43 (30.94)	
Disease stage					0.051
I–II	71 (27.31)	28 (39.44)	16 (22.54)	27 (38.03)	
III–IV	189 (72.69)	58 (30.69)	73 (38.62)	58 (30.69)	

Variables	Total no. (%)	Low no. (%)	Mid no. (%)	High no. (%)	p value
Tumor grade					**0.048**
1	28 (10.77)	15 (53.57)	6 (21.43)	7 (25.00)	
2–3	232 (89.23)	71 (30.60)	83 (35.78)	78 (33.62)	
Histologic type					0.40
Non-serous	143 (54.58)	52 (36.36)	46 (32.17)	45 (31.47)	
Serous	119 (45.42)	34 (28.57)	44 (36.97)	41 (34.45)	
Treatment response[a]					0.75
Yes	124 (70.86)	43 (34.68)	41 (33.06)	40 (32.26)	
No	51 (29.14)	15 (29.41)	17 (33.33)	19 (37.25)	

[a]Only 179 patients had information on treatment response.

Table 2

Associations of lncRNAs and *ASAP1* with ovarian cancer survival.

Expression	HR for progression	95% CI	p value	HR for death	95% CI	p value
ASAP1-IT1						
Unadjusted Cox regression model						
Low	1			1		
Mid	1.12	0.74–1.70	0.59	0.77	0.49–1.20	0.24
High	0.93	0.60–1.42	0.72	0.51	0.32–0.82	**0.0057**
Continuous	0.96	0.78–1.19	0.72	0.72	0.57–0.91	**0.0055**
Adjusted Cox regression model[a]						
Low	1			1		
Mid	0.97	0.63–1.47	0.87	0.69	0.44–1.08	0.11
High	1.09	0.71–1.67	0.70	0.56	0.35–0.90	**0.017**
Continuous	1.04	0.84–1.30	0.72	0.74	0.58–0.94	**0.015**
FAM215A						
Unadjusted Cox regression model						
Low	1			1		
Mid	0.72	0.48–1.08	0.11	0.76	0.49–1.18	0.22
High	0.67	0.44–1.02	0.063	0.55	0.34–0.90	**0.017**
Continuous	0.81	0.65–1.01	0.059	0.74	0.59–0.95	**0.016**
Adjusted Cox regression model[a]						
Low	1			1		
Mid	0.74	0.49–1.13	0.17	0.83	0.53–1.31	0.43
High	0.92	0.60–1.41	0.71	0.74	0.45–1.20	0.22
Continuous	0.95	0.76–1.18	0.63	0.86	0.67–1.09	0.21
LINC00472						
Unadjusted Cox regression model						
Low	1			1		
Mid	0.78	0.52–1.18	0.24	0.76	0.48–1.21	0.25

Expression	HR for progression	95% CI	p value	HR for death	95% CI	p value
High	0.76	0.50–1.15	0.19	0.70	0.44–1.11	0.13
Continuous	0.87	0.70–1.07	0.19	0.83	0.66–1.05	0.12
Adjusted Cox regression model[a]						
Low	1			1		
Mid	0.87	0.58–1.33	0.53	0.89	0.55–1.42	0.62
High	0.92	0.60–1.41	0.71	0.88	0.55–1.42	0.61
Continuous	0.96	0.77–1.19	0.70	0.94	0.74–1.19	0.60
ASAP1						
Unadjusted Cox regression model						
Low	1			1		
Mid	1.81	1.17–2.82	**0.0083**	1.11	0.70–1.76	0.66
High	1.59	1.02–2.49	**0.042**	0.79	0.49–1.27	0.32
Continuous	1.24	1.00–1.52	**0.048**	0.89	0.71–1.12	0.31
Adjusted cox regression model[a]						
Low	1			1		
Mid	1.54	0.98–2.39	0.059	0.94	0.59–1.50	0.79
High	1.63	1.04–2.56	**0.034**	0.75	0.47–1.21	0.24
Continuous	1.26	1.02–1.57	**0.036**	0.87	0.69–1.10	0.24

[a] Adjusted for age, disease stage, tumor grade, and histological type.