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Pluripotent factor lin-28 and its homologue lin-28b in epithelial ovarian cancer and their associations with disease outcomes and expression of let-7a and IGF-II

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ARTICLE INFO

Article history:
Received 4 March 2009
Received in revised form 28 April 2009
Accepted 1 May 2009
Available online 26 May 2009

Keywords:
Epithelial ovarian cancer
Lin-28
Let-7
IGF-II
Prognosis

ABSTRACT

Lin-28 and lin-28B are RNA-binding proteins which can block microRNA let-7 maturation and affect the differentiation and proliferation of embryonic stem cells. Lin-28 may also regulate the expression of insulin-like growth factor II (IGF-II). As one of the pluripotent factors involved in making induced pluripotent stem cells (iPS), *lin-28* is considered a potential therapeutic target for cancer treatment. To further understand the role of *lin-28* in cancer, we *analysed* the expression of *lin-28* and its homologue *lin-28B* in *tumour* samples, and evaluated their associations with let-7a maturation, IGF-II expression, disease features and outcomes in 211 patients with primary epithelial ovarian cancer. The analysis showed that both *lin-28* and *lin-28B* were positively correlated with primary and pre-let-7a-3; *lin-28B*, not *lin-28*, was inversely correlated with mature let-7a. A positive correlation was also observed between *lin-28B* and IGF-II expression, while no association was found between *lin-28B* and IGF-I or IGFBP-3. The study further demonstrated that *lin-28B* expression was associated with the risk of disease progression and death; patients with high *lin-28B* had shorter progression-free and overall survival than those with low *lin-28B*. These results seem to support the findings of recent *in vitro* experiments, showing that lin-28 blocks the process of let-7a maturation. Our study also suggests that lin-28B may promote ovarian cancer progression and serve as an *unfavourable* prognostic marker for the disease. The correlation between *lin-28B* and IGF-II indicates that the growth factor may mediate the effect of lin-28B on *tumour* growth.

1. Introduction

Let-7 has been identified as a critical microRNA (miRNA) in the regulation of essential cellular activities.[1,2] This small non-coding RNA can directly and indirectly regulate hundreds of genes, many of which are involved in cell proliferation, differentiation and apoptosis.[2,3] High mobility group A2 (*Hmga2*) and *RAS* are two of the let-7 targets. Let-7 represses their expression through complementarily pairing to the 3′-UTR of their transcripts. Studies have shown that loss of let-7-directed repression of *Hmga2* and *RAS* promotes malignant transformation, whereas inhibition of *Hmga2* and *RAS* through increasing let-7 expression prevents *tumour* progression.[4–6] Reduced let-7 expression has been observed in many

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doi:10.1016/j.ejca.2009.05.003

forms of cancer, including the lung, breast, ovary and colon; low let-7 expression is also associated with poor prognosis of lung cancer.[6–9] Ectopic expression of let-7 substantially reduces lung *tumour* burden.[10]

Catalysed by RNA polymerase II, *let-7* is initially transcribed into a primary let-7 (pri-let-7), which is processed to pre-let-7 in the nucleus by microprocessors involving Drosha and DGCR8 and then to mature let-7 by Dicer and its cofactors in cytoplasm. The abundance of let-7 is regulated by both transcriptional and post-transcriptional mechanisms.[11–14] Epigenetic regulation involving DNA methylation is part of transcription regulation. One of the *let-7* genes, *let-7a-3*, is embedded in a CpG island; heavy methylation of the region is seen in normal lung tissue [11] and in ovarian cancer.[15] DNA methylation and histone deacetylation are believed to inhibit *let-7a* expression in colon cancer cells because treatment of demethylating agents and histone deacetylase (HDAC) inhibitors can increase its expression and result in global changes in transcription profiles and alterations of *tumour* cell behaviours.[11]

Recently, lin-28 and its homologue lin-28B (together, lin-28 *homologues*) have been identified to play a role in the post-transcription regulation of let-7.[12–14] These proteins are found to block the let-7 maturation, leading to accumulation of pri-let-7 and reduction of mature let-7. Lin-28 is one of the four pluripotent factors that can reprogramme human somatic cells into pluripotent stem (iPS) cells.[16] The 25 kDa protein contains several RNA-binding domains, including a cold-shock domain (CSD) and two retroviral-type CCHC zinc finger domains (ZFMs).[17] As a stem cell-related protein, lin-28 is ubiquitously expressed in embryonic stem cells during early embryogenesis. Through the course of human development, lin-28 expression is gradually down-regulated and restricted to a few tissues.[18] Lin-28B has similar molecular structures and biologic functions as to lin-28,[12] and is found to be over-expressed in human hepatocellular carcinoma and to be able to stimulate tumour growth.[19] Given their abilities to make iPS cells and to control the maturation of let-7 which is known to be involved in certain types of cancer, lin-28 *homologues* are considered potential drug targets for cancer treatment.[12] A recent study also suggests that lin-28 may increase the translation of insulin-like growth factor-II (IGF-II),[20] a known mitogenic growth factor involved in cancer development and progression.[21–23] The actual role of lin-28 homologues in cancer, however, remains largely unknown. To elucidate clinical significance of *lin-28* in cancer, we *analysed* the expression of *lin-28* homologues in epithelial ovarian cancer, and assessed their associations with pri-/pre-let-7a-3, mature let-7a and members of the IGF family. The relationships of *lin-28* homologues with disease characteristics and patient survival outcomes were also evaluated in the study.

2. Materials and methods

2.1. Study patients and their clinical features

Fresh *tumour* specimens were collected between October 1991 and February 2000 for a clinical study of epithelial ovarian cancer in the Department of Gynecology and Obstetrics at University of Turin in Italy. The university's ethical review committee approved the study. Of the 211 patients included in the study, 34 (16.1%) had Grade 1 *tumour*, 40 (19.0%) had Grade 2 *tumour* and 137 (64.9%) had Grade 3 *tumour*. Disease stages I–IV, determined according to the criteria of FIGO, were found in 52 (24.6%), 12 (5.7%), 133 (63.0%) and 14 (6.6%) patients, respectively. The average age of patients at surgery was 57.9 years (range: 26–82). The most common histology was serous papillary (40.3%); the remaining ones were endometrioid (19.4%), undifferentiated (17.1%), mucinous (8.5%), clear cell (7.6%), mullerian (6.6%) and other (0.5%). For data analysis, histological types were classified into two groups: serous and non-serous. Most of the patients received post-operative chemotherapy of platinum-based agents (either cisplatin or carboplatin) with or without paclitaxel after cytoreduction surgery. The patients were followed after surgery through June 2001. The median follow-up time was 31 months, ranging from 0.6 to 114 months. Cytoreductive procedure (debulking) and patient response to chemotherapy were described elsewhere.[24,25] Table 1 shows the clinical and pathological features of patients.

2.2. Tissue analysis of molecular markers

Total RNAs were extracted from the *tumour* samples, which contained 80% to 90% tumour cells, with a standard phenol–

Table 1 – Clinical and pathological features of patients.

Variables	N	%
Age (years), mean (range)	208	57.9 (26–82)
Tumour grade	211	
1	34	16.1
2	40	19.0
3	137	64.9
Disease stage	211	
I	52	24.6
II	12	5.7
III	133	63.0
IV	14	6.6
Residual tumour size (cm)	207	
0	91	44.0
>0	116	56.0
Histological type	211	
Serous papillary	85	40.3
Endometrioid	41	19.4
Undifferentiated	36	17.1
Mucinous	18	8.5
Clear cell	16	7.6
Mullerian	14	6.6
Other	1	0.5
Debulking results	208	
Optimal	108	51.9
Suboptimal	100	48.1
Chemotherapy	211	
No	32	15.2
Yes	179	84.8
Platinum-based	178	84.4
Paclitaxel	76	36.0
Platinum + paclitaxel	75	35.5

chloroform method as described *previously*[21]; cDNAs were prepared using the cloned AMV First-Strand cDNA synthesis kit (Invitrogen, Carlsbad, CA). The expression of *lin-28*, *lin-28B*, pri/pre-let-7a-3 and let-7a was *analysed* using real-time PCR. RNU48 was selected as an endogenous control for normalising gene expression in tissue samples, since RNU48 expression is more reliable in tissue than RNU6B.[26] Given the primer design, our PCR method *analysed* pri- and pre-let-7a-3 in the same assay. The primers for *lin-28*, *lin-28B*, pri-/pre-let-7a-3 and RNU48 were 5′-CTC CGT GTC CAA CCA GCA G (*lin-28* forward), 5′-CAC GTT GAA CCA CTT ACA GAT GC (*lin-28* reverse), 5′-TCA TCT CAC GAG TTT GGA GCT G (*lin-28B* forward), 5′-GCT CTT CTC CAC CAC CTT TGC (*lin-28B* reverse), 5′-GGGTGAGGTAGTAGGTTGTATAGTTTGG (pri-/pre-let-7a-3 forward), 5′-AGG AAA GAC AGT AGA TTG TAT AGT TAT CCC A (pri-/pre-let-7a-3 reverse), 5′-AGTGATGATGACCCCAGGTAACTC (RNU48 forward) and 5′-CTG CGG TGA TGG CAT CAG (RNU48 reverse). Levels of *lin-28*, *lin-28B* and pri-/pre-let-7a-3 expression were *analysed* with the SYBR green-based real-time PCR using the Chromo4™ Real-time PCR System (MJ Research Inc., Waltham, MA). In the PCR reaction (20 μl), 1 μl of cDNA template was mixed with 10 μl of 2 × Power SYBR® PCR master mix (Applied Biosystems), 200 nM of paired primers and water. The PCR amplification included initial incubation at 50 °C for 2 min, denaturing at 95 °C for 10 min and 40 cycles of denaturing at 95 °C for 15 s and annealing at 60 °C for 1 min. Melting curves were *analysed* after each run to verify the size of PCR product. Each sample was tested in duplicate, and the analysis was repeated for those with CV over 5%. A Taqman® MicroRNA assay (Applied Biosystems) was used to analyse let-7a expression which was described elsewhere.[15] IGF peptide concentrations were measured with commercial enzyme-linked immunosorbent assays (ELISAs) (Diagnostic System Laboratories, Webster, Texas),[25,27] and their expressions were *analysed* with the SYBR green-based real-time PCR.[21,22]

2.3. Statistical analysis

Expression index (EI) was calculated for *lin-28 homologues*, pri-/pre-let-7a-3 and let-7a, using the formula: $1000 \times 2^{(-\Delta Ct)}$, where $\Delta Ct = Ct_{target\ genes} - Ct_{RNU48}$. Correlations of lin-28 *homologues* with pri-/pre-let-7a-3, let-7a, IGFs and IGFBP-3 were *analysed* with the Spearman correlation coefficient. To analyse the associations of lin-28 homologues with other factors, the expression of *lin-28* homologues was grouped into low, medium and high categories. Since the two molecules had distinct expression, we used two different schemes to classify their categories. For *lin-28*, the categories were developed based on its tertile distribution. For *lin-28B*, since a third of samples had undetectable expression, these samples were classified as low category. Samples with detectable expression were divided into medium and high categories using median as cutoff. Wilcoxon rank sum test was used to compare levels of let-7a expression among different categories of lin-28. The associations of lin-28 homologues with clinical and pathological variables were analysed using the χ^2 test. Survival analysis was performed to assess the associations of lin-28 homologues with the risk of disease progression and death using the Cox proportional hazards regression model and Kaplan–Meier survival curves. All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

3. Results

Our study showed that *lin-28 homologues* and pri-/pre-let-7a-3 were detectable in most of the samples. The expression of *lin-28* was found in 185 (87.7%) samples, and the average expression was 0.24 EI (5th–95th range: 0.01–16.06). *Lin-28B* was detected in 127 (60.2%) *tumours* with an average expression of 0.22 EI (5th–95th range: 0.002–9.32); pri-/pre-let-7a-3 was seen in 184 (87.2%) samples with an average expression of 0.08 EI (5th–95th range: 0.003–5.94). The expression of *lin-28* and *lin-28B* was positively correlated ($r = 0.305$, $p < 0.001$). The correlation between *lin-28* homologues and let-7 expression was *analysed* both numerically and categorically. In numerical analysis (Table 2A), a positive correlation between *lin-28* and pri-/pre-let-7a-3 ($r = 0.566$, $p < 0.001$) was observed, but not between *lin-28B* and pri-/pre-let-7a-3 ($r = 0.080$, $p = 0.249$). *Lin-28B*, however, was inversely correlated with let-7a ($r =$

Table 2A – Spearman correlations between lin-28 homologues, let-7a and IGFs in epithelial ovarian cancer.

Gene/variables	Lin-28			Lin-28B		
	n	Correlation coefficient	*p*	*n*	Correlation coefficient	*p*
Lin-28B	211	0.305	**<0.001**			
Let-7a	211	0.066	0.343	211	-0.207	**0.003**
Pri/pre-let-7a-3	211	0.566	**<0.001**	211	0.080	0.249
IGF-II peptide	209	0.056	0.421	209	0.142	**0.040**
IGF-II mRNA	202	0.091	0.199	202	0.252	**<0.001**
IGF-II P1 mRNA	197	–0.090	0.210	197	0.010	0.893
IGF-II P2 mRNA	197	–0.072	0.315	197	0.091	0.205
IGF-II P3 mRNA	197	0.139	0.051	197	0.183	**0.010**
IGF-II P4 mRNA	197	0.139	0.051	197	0.196	**0.006**
IGF-I peptide	209	–0.014	0.836	209	0.010	0.886
IGF-I mRNA 1	197	0.105	0.141	197	0.095	0.185
IGF-I mRNA 2	197	–0.014	0.842	197	–0.004	0.961
IGFBP-3 protein	209	–0.075	0.278	209	–0.011	0.877
IGFBP-3 mRNA	202	–0.044	0.531	202	–0.091	0.199

Table 2B – Associations of lin-28 homologues and let-7a expressions in epithelial ovarian cancer.

Variables	Let-7a		Pri/pre-let-7a-3	
	n	Medium (5th–95th)	*n*	Medium (5th–95th)
Lin-28				
Low	70	4.62 (0.39–29.36)	70	0.02 (0–0.27)
Medium	71	4.30 (0.63–30.19)	71	0.05 (0–0.28)
High	70	4.68 (0.79–49.04)	70	0.40 (0–26.92)
p		0.696		**<0.001**
Lin-28B				
Low	84	6.81 (0.79–45.1)	84	0.05 (0–8.32)
Medium	64	3.31 (0.48–27.0)	64	0.02 (0–0.38)
High	63	3.95 (0.70–25.0)	63	0.12 (0–4.32)
p		**0.002**		**<0.001**

–0.207, $p = 0.003$); a similar correlation was not seen between *lin-28* and let-7a ($r = 0.066$, $p = 0.343$).

Wilcoxon analyses showed that the median levels of pri-/pre-let-7a-3 were significantly higher in patients with high *lin-28* or *lin-28B* compared to those with low *lin-28* or *lin-28B* (Table 2B). The expression levels of pri-/pre-let-7a-3 in patients with high, medium and low *lin-28* were 0.40 EI, 0.05 EI and 0.02 EI, respectively ($p < 0.001$). Levels of pri/pre-let-7a-3 were also higher in those with high *lin-28B* than in those with low *lin-28B* (0.12 EI versus 0.05 EI, $p < 0.001$). Let-7a expression was not different by lin-28 expression, but by *lin-28B*. High *lin-28B* had low let-7a, and low *lin-28B* had high let-7a (3.95 EI versus 6.81 EI, $p = 0.002$).

Since lin-28 has been reported to affect IGF-II expression in both normal and cancerous cells (skeletal myoblasts and embryonic carcinoma cell line P19),[20] and high IGF-II expression, especially those from the P3 and P4 foetal promoters, were found in our previous study to be associated with ovarian cancer progression,[21,22] we *analysed* the relationship of *lin-28* and IGFs. Spearman correlation coefficients were calculated for *lin-28* homologues with IGF-I, IGF-II and IGFBP-3 (Table 2A). The results showed that *lin-28B* expression was significantly correlated with IGF-II levels which included peptide concentrations, messenger RNA expression and promoter-specific transcripts (P3 and P4); their correlation coefficients were 0.142 ($p = 0.040$), 0.252 ($p < 0.001$), 0.183 ($p = 0.010$) and 0.196 ($p = 0.006$), respectively. No correlations were found between *lin-28B* and IGF-I or IGFBP-3. *Lin-28* was not related to any of the IGF markers.

To assess the role of *lin-28 homologues* in epithelial ovarian cancer, we first examined their associations with clinical and pathological features of the disease. As shown in Table 3, *lin-28* expression seemed to differ only by histological type ($p = 0.043$), but the difference did not show any clear trend. Associations of *lin-28* with other clinical and pathological variables were not observed (Table 3). *Lin-28B* expression, however, was significantly associated with residual *tumour* size ($p = 0.022$) and debulking results ($p = 0.032$) (Table 3); patients with larger residual *tumours* or suboptimal debulking results tended to have higher *lin-28B* than those with either smaller residual *tumours* or optimal debulking results. Disease stage and *tumour* grade were not associated with *lin-28B* expression. These results basically indicated clinical and pathological factors having little impacts on the expression of *lin-28* homologues in epithelial ovarian cancer.

We further evaluated the associations of *lin-28* homologues with disease outcomes. Kaplan–Meier survival analysis showed substantial differences in disease progression-free survival and overall survival among patients with different levels of *lin-28B* expression (Fig. 1). Patients with high *lin-28B* had shorter progression-free and overall survival compared to those with low *lin-28B* ($p = 0.0017$ and $p = 0.0022$, respectively). These associations, however, were not observed for *lin-28* (data not shown). To evaluate if the effect of *lin-28B* on survival was independent from clinical and pathological variables, we *analysed* the survival data using the Cox propor-

Table 3 – Associations of lin-28 homologues with clinical and pathological variables in epithelial ovarian cancer.

Variables	N	Lin-28				Lin-28B			
		Low	Medium	High	*p*	Low	Medium	High	*p*
Tumour grade					0.832				0.123
1–2	74	26 (35.1)	23 (31.1)	25 (33.8)		36 (48.7)	21 (28.3)	17 (23.0)	
3	137	44 (32.1)	48 (35.0)	45 (32.9)		48 (35.0)	43 (31.4)	46 (33.6)	
Disease stage					0.401				0.058
I–II	64	23 (35.9)	24 (37.5)	17 (26.6)		31 (48.4)	21 (32.8)	12 (18.8)	
III–IV	147	47 (32.0)	47 (32.0)	53 (36.0)		53 (36.0)	43 (29.3)	51 (34.7)	
Residual size (cm)[a]					0.455				**0.022**
0 cm	91	31 (34.1)	34 (37.4)	26 (28.6)		44 (48.3)	29 (31.9)	18 (19.8)	
>0 cm	115	37 (32.2)	36 (31.3)	42 (36.5)		39 (33.9)	34 (29.6)	42 (36.5)	
Histological type					**0.043**				0.106
Non-serous	126	49 (38.9)	35 (27.8)	42 (33.3)		57 (45.2)	37 (29.4)	32 (25.4)	
Serous	85	21 (24.7)	36 (42.4)	28 (32.9)		27 (31.7)	27 (31.8)	31 (36.5)	
Debulking results[a]					0.562				**0.032**
Optimal	108	39 (36.1)	36 (33.3)	33 (30.6)		51 (47.2)	33 (30.6)	24 (23.2)	
Suboptimal	99	29 (29.3)	35 (35.4)	35 (35.4)		32 (32.3)	30 (30.3)	37 (37.4)	

a Not all subjects had the information.

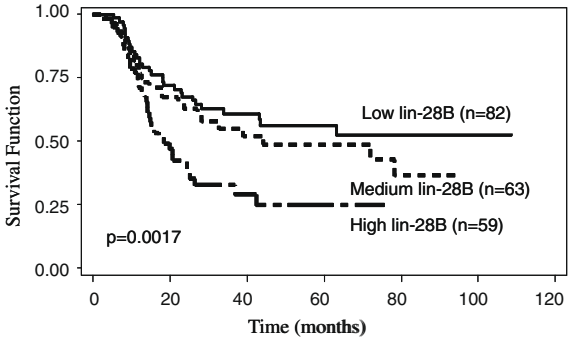

Fig. 1A – Kaplan–Meier disease progression-free survival curves by levels of *lin-28B* expression.

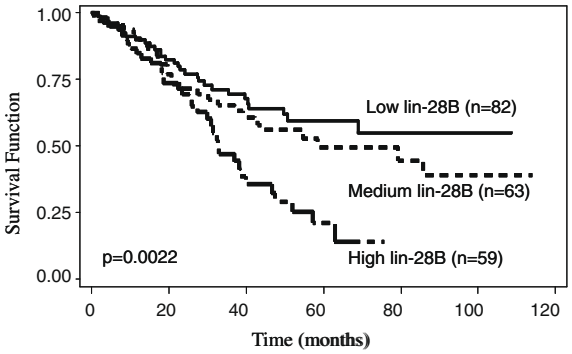

Fig. 1B – Kaplan–Meier overall survival curves by levels of *lin-28B* expression.

tional hazards regression model. Both univariate and multivariate regression analyses showed that high *lin-28B* was significantly associated with increased risk of disease progression and death, and the associations remained significant after patient's age at surgery, disease stage, *tumour* grade, *tumour* histology and residual *tumour* size were adjusted in the analysis (Table 4). Patients with high *lin-28B* had 2-fold increases in risk of disease progression (hazard ratio (HR) = 2.00, 95% confidence interval (CI): 1.22–3.29) and death (HR = 2.10, 95% CI: 1.26–3.53) compared to those with low *lin-28B* after the adjustment. The survival analysis also indicated that there was a dose-dependent relationship between *lin-28B* expression and risk of disease progression or death ($p = 0.007$ and 0.006, respectively). The Kaplan–Meier survival analysis was repeated among patients with stage II, III or IV diseases, and the results were similar (data not shown). We also performed additional Cox regression analysis only among the patients who had detectable lin-28B expression. The associations remained significant for overall survival, but were less significant for progression-free survival, probably due to a smaller sample size (data not shown).

4. Discussion

Several recent studies showed that the pluripotent factor lin-28 and its homologue lin-28B were able to interfere with let-7 maturation.[12–14,28] Using biochemical approaches, Viswanathan and colleagues[12] discovered that lin-28 was a RNA-binding protein which could interact with microprocessor Drosha, selectively blocking the process of let-7 maturation, which led to accumulation of pri-/pre-let-7. The authors also found that lin-28B, which was over-expressed in human hepatocellular carcinoma and several cancer cell lines,[19] had a similar function to block let-7 processing. Experiments further indicated that the blockage of let-7 maturation by lin-28 occurred when lin-28 bound to the hairpin structure of let-7[13,14]; blocking the binding of lin-28 to let-7 could restore the process.[13] However, findings of the relationship between lin-28 and pre-let-7 were inconsistent. Rybak and colleagues[29] reported that lin-28 interfered with Dicer in pre-let-7 processing, leading to the accumulation of pre-let-7, while another group showed that lin-28 induced the degradation of uridylated pre-let-7.[28] We found that high expression of *lin-28* and *lin-28B* was associated with high levels of let-7a precursors, pri- and pre-let-7a-3. To

Table 4 – Associations of lin-28b expression with risk of disease progression or death in epithelial ovarian cancer.

Lin-28B	Progression		Death	
	HR[a]	95% CI[b]	HR	95% CI
Univariate analysis				
Low	1.00		1.00	
Medium	1.30	0.78–2.16	1.30	0.77–2.18
High	2.32	1.43–3.78	2.33	1.41–3.83
Test for trend	**<0.001**		**0.001**	
Multivariate analysis				
Medium lin-28B (versus low)	1.17	0.70–1.96	1.13	0.67–1.92
High lin-28B (versus low)	2.00	1.22–3.29	2.10	1.26–3.53
Age	1.01	0.99–1.03	1.00	0.98–1.03
Disease stage	1.48	1.07–2.04	1.43	1.01–2.04
Tumour grade	1.34	0.92–1.95	1.40	0.91–2.13
Residual tumour size	1.13	1.04–1.21	1.26	1.17–1.36
Serous (versus non-serous)	1.32	0.85–2.04	0.74	0.48–1.15
Trend test for lin-28B	**0.007**		**0.006**	

a HR: Hazards ratio obtained from the Cox proportional hazards regression analysis.
b CI: Confidence interval obtained from the Cox proportional hazards regression analysis.

our knowledge, this is the first human study to show that *lin-28* and let-7a precursors are correlated in *tumour* tissue.[12] However, our results were not entirely consistent with the experimental findings. We did not observe an inverse correlation between mature let-7a and *lin-28* expression, although it was suggested for *lin-28B*. This discrepancy may suggest different miRNA processing in embryonic cells as oppose to ovarian epithelial cells, or cell-specific actions of lin-28 homologues. The influence between lin-28 and let-7 may be reciprocal. A recent study showed that let-7 and miR-125 could regulate the activity of *lin-28* by reducing its expression.[29] Vasudevan and colleagues reported that let-7 could activate rather than repress the expression of target genes under growth-arrest conditions.[30] The regulatory feedback loop and cell cycle-dependent regulation may make it difficult to consolidate the findings of *in vivo* and *in vitro* studies on *lin-28*.

In the study, we found that compared to those with low *lin-28B*, patients with high *lin-28B* had higher IGF-II expression at both mRNA and peptide levels. These findings suggest that lin-28B may either indirectly influence the growth factor through its regulation of other molecules, or directly regulate its activity.[20,31,32] A previous study showed that lin-28 could bind to IGF-II mRNA to enhance its translation in muscle cells.[20] If this is true in ovarian cancer, then IGF-II may have a role in mediating the effect of lin-28B. High levels of IGF-II expression have been observed in aggressive ovarian cancer and are associated with poor prognosis of the disease.[21–23] Temporal elevation of *lin-28* and IGF-II was also seen during the development of *Caenorhabditis elegans*,[33] suggesting that *lin-28* and IGF-II may be integrated in response to growth signals. Interestingly, *lin-28* is not correlated with IGF-II expression in our study, although lin-28 homologues have similar molecular structures and functions.[14] It is unclear why *lin-28B*, not *lin-28*, correlates with IGF-II expression in epithelial ovarian cancer. Tissue-specific effect of lin-28 on IGF-II may be a possible explanation for the difference between lin-28 and lin-28B. The relationship between *lin-28B* and IGF-II is quite compelling in our study because it is observed at both mRNA and peptide levels. Moreover, this relationship seems to be unique to IGF-II because no correlations are found between *lin-28* homologues and IGF-I or IGFBP-3. Our observation of *lin-28* and IGF-II correlation seems to support the notion that some lin-28 actions are mediated through IGF-II.[20]

The study suggests that lin-28B may act in *favour* of ovarian cancer progression, leading to poor prognosis. This finding is in agreement with the observation of Guo and colleagues, who found that *lin-28B* over-expression could stimulate *tumour* growth.[19] Our results also support the findings of two other studies.[8,9] Shell and colleagues[9] reported that loss of let-7 induced *Hmga2* expression, which was associated with *unfavourable* prognosis of ovarian cancer. Similarly, in lung cancer reduced let-7 expression was associated with short post-surgical survival.[8] Let-7 was found to be able to inhibit cancer progression and tumourigenesis by maintaining cell differentiation and suppressing *RAS* and *Hmga2* expression.[5,6,9] Aberrant miRNA processing could accelerate oncogenic transformation.[34] Very recently, Chang and colleagues[35] demonstrated that lin-28B mediated c-Myc-driven cell proliferation and let-7 repression. Additionally, IGF-II may also be involved in the lin-28B-related *tumour* progression. We have found that IGF-II is significantly associated with ovarian cancer progression and patient survival.[21,22] Moreover, our previous study also suggests that IGF-II may interact with let-7a in epithelial ovarian cancer.[15] These results, however, seem to be inconsistent with the report by Brueckner and colleagues,[11] who have found that ectopic expression of let-7a-3 in lung cancer cell line A549 may increase oncogenic characteristics. This discrepancy may suggest the possibility of diverse biological actions in different subclasses of let-7. Our study is the first clinical investigation to show that lin-28B may play an important role in ovarian cancer progression, and assessing *lin-28B* expression in ovarian *tumour* may help to predict patient prognosis. The discrepancy between lin-28 and lin-28B with regard to their association with ovarian cancer progression may indicate tissue-specific or developmental stage-dependent actions. If our finding of *lin-28B* in ovarian cancer can be confirmed by larger studies, this molecule may have the potential to serve not only as a prognostic marker but also as a therapeutic target given its role in stem cell renewal.

In summary, we found some correlations between *lin-28* homologues and let-7a in epithelial ovarian cancer, which support the notion that lin-28 may interfere the process of let-7a maturation. A positive association was also observed between *lin-28B* and IGF-II expression, which may explain the adverse effect of *lin-28B* on ovarian cancer survival. High *lin-28B* expression was found to be associated with unfavourable disease outcomes. These findings suggest that lin-28B may be involved in ovarian cancer progression through its regulatory influence on miRNA processing and IGF-II expression.

Conflict of interest statement

None declared.

Acknowledgements

K. Shaverdashvili was supported by a UICC International Cancer Technology Transfer Fellowship and with Federal funds from the National Cancer Institute, National Institutes of Health under Contract NO2-CO-41101.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2009.05.003.

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