Letter

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Potential proteins targeted by let-7f-5p in HeLa cells

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Summary **MicroRNAs are a class of small, endogenous, non-coding RNAs mediating posttranscriptional gene silencing. The current authors hypothesized that let-7f-5p is likely involved in cell invasion and proliferation by regulating the expression of target genes. The current study combined let-7f-5p with iTRAQ to assess its effect on gene expression in HeLa cells. Results indicated that 164 proteins were expressed at different levels in HeLa cells overexpressing let-7f-5p and negative controls and that 172 proteins were expressed at different levels in let-7f-5p-silenced HeLa cells and negative controls. Results indicated that let-7f-5p may suppress insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) in HeLa cells.**

Keywords: Proteomic analysis, let-7f-5p, IGF2BP1, HeLa cells

MicroRNAs (miRNAs) are a class of endogenous, non-protein coding RNAs that are small (approximately 22 nucleotides in length) and highly conserved. MiRNAs have a widespread impact on regulation of gene expression and evolution and are thought to affect over 50% of all human genes (*1*). let-7 miRNA was originally identified in *Caenorhabditis elegans* (*C. elegans*) as a regulator of developmental timing and cell proliferation (*2*). The let-7 family is a particularly interesting example as one of the few families that are also conserved in *Drosophila* and *C. elegans*. In humans, the let-7 family consists of 9 mature let-7 miRNAs encoded by 12 different genomic loci, some of which are clustered together.

let-7f, which is a member of the let-7 family, is located at 9q22.3. More importantly, let-7f is a novel regulator in human endocervical cells and is involved in the induction of immune tolerance (*3*). let-7f was found to play an important role in cell growth, migration, invasion, and angiogenesis in tumors (*4*). The aim of the current study was to investigate the relationship between let-7f-5p and the genes it potentially targets at the protein level *in vitro*.

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Five thousand and fifty-two proteins were identified from 31,666 peptides at a minimum confidence level of 95%. Results identified 164 proteins that were expressed at significantly different levels in HeLa cells overexpressing let-7f-5p, including 59 proteins that were up-regulated (1.5-fold, $p < 0.5$) and 105 proteins that were down-regulated (1.5-fold, $p < 0.5$). One hundred and seventy-two proteins were identified in let-7f-5p-inhibited HeLa cells, including 44 proteins that were up-regulated (1.5-fold, $p < 0.5$) and 128 proteins that were down-regulated (1.5-fold, $p < 0.5$). Expression of IGF2BP1, vimentin, Keratin, and Protein FAM decreased while expression of Integrinα1 increased in HeLa cells overexpressing let-7f-5p. In let-7f-5p-silenced HeLa cells, expression of IGF2BP1 and Integrin α1 increased while expression of vimentin and T-complex protein decreased. KEGG analysis revealed that 4 biological pathways including arrhythmogenic right ventricular cardiomyopathy, pyrimidine metabolism, RNA degradation, and the pentose phosphate pathway differed significantly in HeLa cells overexpressing let-7f-5p and that three pathways including glycolysis, alanine, aspartate and glutamate metabolism, and the spliceosome pathway differed significantly in let-7f-5p-silenced HeLa cells.

Study data revealed that let-7f-5p overexpression dramatically suppressed *IGF2BP1* and *vimentin*, thus possibly regulating cell migration and invasion *in vitro*. Moreover, let-7f-5p inhibitors significantly upregulated the expression of IGF2BP1 (Table 1). Vimentin and keratin are markers of cell proliferation and invasion,

Table 1. Proteins expressed at different levels in HeLa cells transfected with a let-7f mimic and an inhibitor

Gene symbol	Protein	Molecular function	Let-7f mimic/control -fold change	*P* value	Let-7f inhibitor/control -fold change	*P* value
RPS4X	ribosomal protein S4, X-linked	poly(A) RNA binding	2.188	0.026	0.433	0.008
ADARB1	adenosine deaminase, RNA-specific, B1	RNA binding	1.722	0.020	1.600	0.014
MAN2A1	mannosidase, alpha, class 2A, member 1	carbohydrate binding	2.188	0.035	1.675	0.046
BZW1	basic leucine zipper and W2 domains 1	poly(A) RNA binding	0.525	0.018	0.515	0.010
CCDC6	coiled-coil domain containing 6	SH3 domain binding	0.555	0.037	0.377	0.031
HNRNPU	heterogeneous nuclear ribonucleoprotein U	ATP binding	3.020	0.026	2.443	0.004
HIST1H4A	histone cluster 1, H4a	poly(A) RNA binding	0.074	0.010	2.109	0.004
IGF2BP1	insulin like growth factor 2 mRNA binding protein 1	mRNA binding	0.525	0.020	2.858	0.009
ITGA1	integrin subunit alpha 1	collagen binding	2.291	0.039	2.630	0.042
ITSN2	intersectin 2	SH3 /SH2 adaptor activity	0.308	0.018	0.608	0.040
NFRKB	nuclear factor related to kappaB binding protein	protease binding	0.575	0.019	0.083	0.037
PLEC	plectin	poly(A) RNA binding	0.631	0.030	0.145	0.000
VAC14	Vac14 homolog	protein binding	0.096	0.036	0.013	0.019
RMND1	required for meiotic nuclear division 1 homolog	protein binding	0.305	0.004	5.598	0.025
RPIA	ribose 5-phosphate isomerase A	protein binding	0.305	0.031	0.156	0.018
DDB1	damage-specific DNA binding protein 1	DNA binding	1.629	0.048	0.525	0.041
PRKDC	protein kinase, DNA activated, catalytic polypeptide	DNA-dependent protein kinase activity	1.977	0.000	3.698	0.000
ESRP2	epithelial splicing regulatory protein 2	mRNA binding	0.011	0.019	0.011	0.019
CSE1L	CSE1 chromosome segregation 1-like	nuclear export signal receptor activity	2.070	0.030	0.525	0.047
FKBP15	FK506 binding protein 15	protein binding	0.157	0.018	0.631	0.039
HSP90AA1	heat shock protein 90kDa alpha family class A member 1	ATP binding	2.421	0.017	0.535	0.011
KNTC1	kinetochore associated 1	protein binding	2.270	0.049	3.281	0.015
MED16	mediator complex subunit 16	protein binding	0.095	0.037	0.179	0.080
MYH9	myosin, heavy chain 9, non-muscle	ATP binding	0.394	0.000	0.127	0.000
NOA1	nitric oxide associated 1	GTP binding	1.542	0.040	1.675	0.042
SRRM2	serine/arginine repetitive matrix 2	poly(A) RNA binding	0.592	0.000	0.331	0.016
RIOK1	RIO kinase 1	protein binding	0.104	0.018	31.333	0.049
TOX4	TOX high mobility group box family member 4	protein binding	0.142	0.003	0.265	0.003
VIM	vimentin	protein binding	0.619	0.020	0.334	0.002
ZNF784	zinc finger protein 784	DNA binding	0.104	0.018	0.104	0.019

and let-7f-5p mimics decreased the levels of vimentin and keratin protein.

IGF2 mRNA binding protein 1 is a member of the RNA-binding IGF2BP protein family, and 3 members of that family are found in mammals (IGF2BP1/2/3) (*5*). To the extent known, *IGF2BP1* is exclusively expressed during embryogenesis but is synthesized *de novo* in a broad variety of malignancies (*6*). The overexpression of *IGF2BP1* not only enhances the velocity of cell motility but also promotes the directionality of migration (*7*). A high level of *IGF2BP1* expression enhances the migratory and invasive potential of cells and promotes their proliferation (*8*). *IGF2BP* family members are essential for the migration of neural crest cells and the central regulation of the properties of stem cells within the LIN28/Let-7 networks (*9,10*). In the current study, expression of IGF2BP1 decreased while expression of Integrinα1 increased in HeLa cells overexpressing let-7f-5p. In let-7f-5p-silenced HeLa cells, expression of IGF2BP1 and Integrinα1 increased.

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References

1. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: Contributions of translational repression and mRNA decay. Nat Rev Genet. 2011; 12:99-110.
2. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature. 2000; 403:901-906.
3. Sathe A, Patgaonkar MS, Bashir T, Reddy KV. MicroRNA let-7f: A novel regulator of innate immune response in human endocervical cells. Am J Reprod Immunol. 2014; 71:137-153.
4. Liang S, He L, Zhao X, Miao Y, Gu Y, Guo C, Xue Z, Dou W, Hu F, Wu K, Nie Y, Fan D. MicroRNA let-7f inhibits tumor invasion and metastasis by targeting MYH9 in human gastric cancer. PloS One. 2011; 6:e18409.
5. Yisraeli JK. VICKZ proteins: A multi-talented family of regulatory RNA-binding proteins. Biol Cell. 2005; 97:87-96.
6. Yaniv K, Yisraeli JK. The involvement of a conserved family of RNA binding proteins in embryonic development and carcinogenesis. Gene. 2002; 287:49-54.
7. Stohr N, Kohn M, Lederer M, Glass M, Reinke C, Singer RH, Huttelmaier S. IGF2BP1 promotes cell migration by regulating MK5 and PTEN signaling. Genes Dev. 2012; 26:176-189.
8. Gutschner T, Hammerle M, Pazaitis N, *et al*. Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is an important protumorigenic factor in hepatocellular carcinoma. Hepatology. 2014; 59:1900-1911.
9. Farina KL, Huttelmaier S, Musunuru K, Darnell R, Singer RH. Two ZBP1 KH domains facilitate beta-actin mRNA localization, granule formation, and cytoskeletal attachment. J Cell Biol. 2003; 160:77-87.
10. Nishino J, Kim S, Zhu Y, Zhu H, Morrison SJ. A network of heterochronic genes including Imp1 regulates temporal changes in stem cell properties. ELife. 2013; 2:e00924.

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