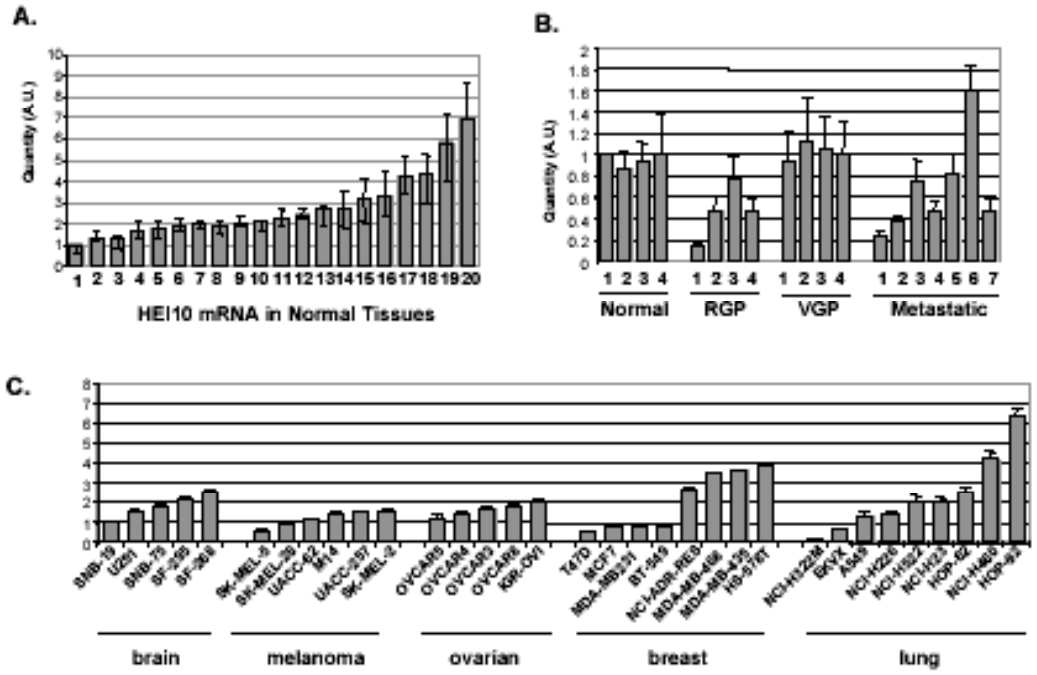
Gene	Role	Sequence
CDC2(Cdk1)		Assay-on demand set for Hs00364293_m1
Cyclin B1	F	ATGGCAGTGACACCAACCA
	R	TTATTGATCGGTTCATGCAGAA
	P	TGCAGCATCTTCTTGGGCACACA
Cyclin B2	F	GGCATTATGGATCGATTTTTACAG
	R	GAGCAGAGCAGTAATCCCAACTAA
	P	TCAGCCAGTTTCCCGGAAGAAGCTT
FAK	F	GATGGCTCCAGAGTCAATCAATT
	R	CACATACCACACCAAACATCCATA
	P	TCGACGTTTTACCTCAGCTAGTGAC
HEI10	F	AGAGACGTTCCTGATGTACCTGAT
	R	AGCTATGGTATTGGCAGGACTG
	P	TCGGGAGCTAATGTCCAACACGATCTCT
NF2	F	CTCCTTCCCGTGGAATGAA
	R	TGAGGAGTTAAACTTGAAGACATCAAT
	P	TCCGAAACATCTCGTACAGTGACAAGGAG
p130cas	F	CAGCCAGTATGGCCAGGA
	R	GGTCTCGGCCATTGGG
	P	ACACACCCCCCATGGCTGTCAA
paxillin	F	GGAGCTGGACGAGCTGATG
	R	GGTGAGCTGCTCCCTGTCT
	P	TCGCTGTCGGATTTCAAGTTCATGG
polR2F	F	TGCCATGAAGGAACTCAAGG
	R	TCATAGCTCCCATCTGGCAG
	P	CCCCATCATCATTCGCCGTTACC

Supplemental Table 1. **Primer and probe sequences used in RTq-PCR, and related methods**. Taqman sets were designed using Primer Express™ version 2.0 software from Applied Biosystems. All sequences are given 5' to 3'. F: forward primer. R: reverse primer. P: probe. The 5'- and 3' ends of the probes were labeled with the reporter dye 6-FAM (6-carboxy-

fluorescein) (Glenn Research) and the quencher dye BHQ1 (Black Hole Quencher) (Biosearch Technologies) respectively. All primers and probes were synthesized by the FCCC Fannie Rippel Biotechnology facility. PCR master mix from Eurogentec was used for PCR. The concentrations of primers and probes were 400 nM and 100 nM respectively. For CDK1 (CDC2), an Assay-on-Demand from Applied Biosystems was used (sequence reference in Table 1) in combination with Universal Master mix. Cycling conditions were 95°C, 15 min followed by 40 (2-steps) cycles (95°C, 15 sec; 60°C, 60 sec). Ct (cycle threshold) values were converted to quantities (in arbitrary units) using a standard curve (4 points, 5-fold dilutions) established with a calibrator sample. For each sample, 2 RT reactions were performed with inputs of 50 and 10 ng. An aliquot of the cDNA was used for PCR. The values plotted on graphs in the manuscript are the average from 2 PCR reactions after adjustment for input.



Singh et al., Supplemental Figure 1

Supplementary Figure 1, Legend. Distribution of HEI10 in cell lines and primary tissues. **A**. Total RNA from 20 human tissue types, represented by 3 independent samples each, was analyzed by qRT-PCR to establish the relative abundance of HEI10. Scale shown reflects relative ratios for each transcript, expressed in arbitrary units. Tissues assessed included 1, skeletal muscle' 2, placenta; 3, prostate; 4, liver; 5, thymus; 6, spleen; 7, brain; 8, small intestine; 9, colon; 10, adipose; 11, cervix; 12, trachea; 13, bladder; 14, esophagus; 15, ovary; 16, lung; 17, thyroid; 18, kidney; 19, testes; 20, heart. **B**. Total RNA prepared from normal melanocyte cell lines, and from melanoma cell lines from the Herlyn laboratory/Wistar Institute melanoma resource(Satyamoorthy et al., 2003) was quantified using the Agilent Bioanalyzer, then was analyzed by RT-qPCR. Data shown for Melanocyte cell lines are 1, HeMn-MP (50% confluent); 2, HeMn-MP (100% confluent); 3, AM003104; 4, HeMn-LP; for RGP melanomas, 1, WM35; 2, WM3211; 3, WM793; 4, SBCL12; for VGP melanomas, 1, WM1366; 2, WM115; 3, WM3248; 4, WM278; for metastastic melanomas, 1, 1205Lu; 2, WM9; 3, 451Lu-; 4, WM1158; 5, WM164; 6, WM293A; 7, WM1232. **C**. The cancer cell lines indicated from the NCI reference panel(Weinstein et al., 1997) were analyzed as in **B** to establish the relative abundance of HEI10 mRNA.

Supplemental Figure 1, Methods. Overexpression of the HEI10 mRNA has been reported by one group to specifically correlate with melanoma cell metastasis (Smith et al., 2004). Our data imply a complex function of HEI10 that does not immediately suggest a pro-metastatic action, as reduction of HEI10 inhibits cell proliferation, but increases motility. Moreover, our own experiments with cultured cells, and analysis of HEI10 transcripts in NCBI public resources such as the Gene Expression Omnibus (Wheeler et al., 2004), instead implied that the HEI10 mRNA might be more broadly expressed.

To better understand HEI10 distribution, we used RT-qPCR to analyze the relative abundance of HEI10 mRNA across 20 human tissue samples (Supplemental Figure 1A). Ambion's First Choice ® human total RNA survey panel was used as a source of RNA from 20 different normal tissues. Each sample, provided at 1 mg/ml, is a pool of RNA from at least 3 donors. HEI10 was readily detected in all tissues analyzed, with no more that ~6-fold variation between highest (heart) and lowest (skeletal muscle) HEI10-expressing tissues.

We next compared HEI10 expression in human melanocytes, and in cell lines derived from radial growth phase (RGP), vertical growth phase (VGP) and metastatic melanomas (Supplemental Figure 1B), from a panel of cell lines generously provided by Dr. Meenhard Herlyn from his Resource Panel maintained at the Wistar Institute. Normal human neonatal

melanocyte cell lines (HeMn-LP, HeMn-MP) were obtained from Cascade Biologics. A primary adult human melanocyte cell line (AM033104) was established from excess skin obtained from a surgical procedure under IRB 00-821 at Fox Chase Cancer Center. No clear trend of HEI10 expression emerged in comparing these cell lines. Although lowest levels of HEI10 were observed in RGP cell lines, normal cells and VGP cell lines had comparable levels of HEI10 mRNA that were only 2-fold higher than RGP levels.

For comparison, we also investigated HEI10 expression in a set of cancer-derived cell lines from the NCI reference panel ((Suggitt & Bibby, 2005), and references therein). RNA was provided by the FCCC Translational facility. This analysis indicated that HEI10 showed little variation of expression between brain, melanoma, and ovarian cancer cell lines (Supplemental Figure 1C). However, interestingly, breast tumors divided into two clear groups marked by low and high expression HEI10, with the latter group characteristically having 4-6 fold higher steady state levels. The lung cancer cell lines also showed substantial variability of HEI10 expression, with 30-fold difference between the highest (HOP-92) and lowest (NCI-H322M) HEI10-expressing cell lines.

Finally, as HEI10 functions in control of cell cycle (Toby et al., 2003), we considered that abundance of the HEI10 transcript might in turn be cell cycle-regulated (as is cyclin B (Alvarez et al., 2001)), or regulated in response to contact inhibition (relevant to interaction with Merlin (Edgar, 2006; Morrison et al., 2001)). We contrasted HEI10 transcript abundance in 10 different cell lines at ~50% confluence (actively proliferating), versus in cells maintained under conditions of contact inhibition for 2 days. Based on this analysis (results not shown), HEI10 expression was essentially equivalent in contact-inhibited, non-proliferating cells and in actively proliferating cells (results not shown), implying this means of regulation did not apply.

Culture conditions for all cells are available on request. Upon harvest, cell pellets were collected and stored at -80ºC until processed for RNA preparation, which was done using an RNeasy kit (Qiagen).

Supplemental Figure 1, References.

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