Published in final edited form as:

Pigment Cell Melanoma Res. 2010 December ; 23(6): 845–848. doi:10.1111/j.1755-148X.2010.00753.x.

Quantitative measurement of circulating lymphoid-specific helicase (*HELLS*) gene transcript: a potential serum biomarker for melanoma metastasis

Hye-Eun Kim, James T. Symanowski, Erika E. Samlowski, Jason Gonzales, and Byungwoo Ryu

Section of Melanoma, Renal Cancer and Immunotherapy, Nevada Cancer Institute, One Breakthrough Way, Las Vegas, NV, USA

Dear Sir,

Metastasis of tumor from a primary site to distant organs poses a significant medical problem and is the leading cause of human cancer deaths. Metastatic tumors are highly refractory to therapeutic treatments, resulting in poor clinical outcomes. For example, early melanoma patients, AJCC (American Joint Committee on Cancer) Stage I and II, have a 10-yr survival rate of approximately 90%, whereas the 5-yr survival rate for AJCC Stage IV patients is <5% (Balch et al., 2009). Histologic examination of early melanoma lesions is difficult and cannot always predict the aggressiveness of the melanoma (Cook et al., 2002). Breslow thickness and ulceration remain the most powerful prognostic factors for primary melanomas. However, these features are not completely accurate biological indicators of malignant potential, as a subgroup of patients with a thin localized melanoma (<2.0 mm) will eventually develop metastases (Becker et al., 2006). The discovery of reliable biomarkers that could identify melanoma patients with metastasis as early as possible for adjuvant treatment would contribute to increased survival.

Circulating nucleic acid (CNA) consists of extracellular genetic material (DNA and RNA) found freely in human body fluids. These information-rich molecules of tumor-derived CNAs have a great potential as a noninvasive source of candidate biomarkers because they can be used to monitor the status of remote tumors by decoding the contained genetic and epigenetic information, potentially avoiding the need for tissue biopsies (Swarup and Rajeswari, 2007). One of the most important factors determining the potential value of CNA as a predictive biomarker is the molecular information intrinsically encoded in the target CNAs. For example, genetic/epigenetic changes in genes specific for the clinicopathologic characteristics of tumor, as well as differential levels of circulating mRNAs from genes that have functional roles in the malignant progression, would be near ideal CNA candidates as a source of predictive biomarkers.

Using a global gene expression profiling technique, we previously discovered a gene signature associated with metastatic phenotype in a panel of melanoma cell lines derived

© 2010 John Wiley & Sons A/S

Correspondence Byungwoo Ryu, bryu@nvcancer.org.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Materials and Methods.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

from histopathologically well-characterized lesions of melanomas (Ryu et al., 2007). Dr. Winnepenninckx et al. (2006) also reported a 254-gene signature associated with high-risk of recurrent metastasis and poor clinical outcomes in a study using human tissue samples. A significant portion of these gene signatures overlapped. Common molecular pathways mediated by these signature genes in metastatic melanomas involve the activation of DNA replication origins, DNA repair, and mitogenic proliferation. These findings strongly suggest that one of the distinctive molecular characteristics of metastatic melanoma is the over-expression of the genes associated with these cellular pathways. Therefore, we hypothesized that levels of the metastasis-associated gene transcripts circulating in patient's blood might correlate with the presence of metastasis and could potentially be utilized as a serum marker for the occurrence of metastases.

We conducted an exploratory study to test this hypothesis. Four genes (*HELLS*, *TYMS*, *NCAPH*, and *BIRC5*) representing the metastasis-associated gene signature were selected. Serum levels of the gene transcripts circulating in patient blood were measured using duplex real-time q-PCR technique (see Appendix S1 for detailed experimental methods). Serum levels of the selected gene transcripts were compared in two groups of melanoma patients; group 1, patients with localized melanomas (AJCC stage 0/1) and group 2, patients with distant organ metastasis (AJCC Stage IV). To establish base levels of the circulating gene transcripts in a normal control population, serum samples from sex- and age-matched healthy controls with no history of illness (average age, 57.8 yr) were included. Key clinical and histopathologic characteristics of the melanoma patients included in this study are summarized in Supporting Information Table S1. As shown in Figure 1A, there was a clear difference in the levels of circulating transcript of three of the selected genes (*HELLS*, *NCAPH*, and *TYMS*) in patients with early stage primary melanomas and patients with distant organ metastasis, but this difference was not seen for *BIRC5*. Serum levels of *ACTB* (β -actin) gene transcript served as a negative control and showed no differences between groups 1 and 2 (Figure 1A). Statistical calculations using analysis of variance ($_{ANOVA}$) demonstrated that the differences were significant for *HELLS* (P-value <0.001), *NCAPH* (P-value <0.001), and *TYMS* (P-value = 0.004), but not for *BIRC5* (P-value = 0.633) or *ACTB* (P-value = 0.918) (Supporting Information Table S2).

To calculate the predictive value of the serum levels of the gene transcripts for metastasis presence, a logistic regression analysis was performed. The probability of metastasis was increased with increasing serum levels of *HELLS* and *NCAPH* but not *TYMS* or *BIRC5*. The odds ratio of *HELLS* (94.2) was greater than that of *NCAPH* (10.5) (Table 1). *HELLS* and *NCAPH* were included in a multiple logistic regression analysis, and backward elimination was used to determine independent predictors for metastasis. Results showed that only *HELLS* was independently predictive of metastasis. Serum lactate dehydrogenase (LDH) is a strong prognostic factor in the AJCC staging system for metastatic melanoma, and may influence treatment outcome (Balch et al., 2009; Deichmann et al., 1999). We compared the probability that the serum levels of the four gene transcripts were metastasis-predicting indicators with the probability of serum LDH level as an indicator by calculating receiver operating characteristic – area under curve (ROC-AUC) values (Figure 1B, C). The AUC area was greater for *HELLS* (1.0) and *NCAPH* (0.939) than for LDH (0.778). The serum transcript level of *HELLS* had the highest ROC-AUC value (Figure 1C). These findings clearly indicate that measurement of the target gene transcript levels in patient serum had a better discriminatory potential than LDH for detection of metastatic melanoma.

The serum markers S100B and melanoma-inhibiting activity (MIA) have been proposed as prognostic markers for melanoma progression because they have a strong association with overall survival. However, these markers do not have an independent prognostic capability when compared to LDH serum concentration (Deichmann et al., 1999). Therefore, LDH is

currently the only serum marker integrated in the AJCC staging system (Balch et al., 2009). Our study clearly demonstrates that measurement of metastasis-associated gene transcript levels in patient serum is superior to serum LDH concentration in discriminating patients with distant organ metastasis (Figure 1C). This preliminary finding warrants a larger-scale prospective study to validate circulating metastasis-associated gene transcripts, especially *HELLS* gene transcript, as a serum prognostic marker for the risk of metastasis.

HELLS gene encodes a lymphoid-specific helicase, HELLS. HELLS is a member of an SNF2 subfamily of helicases which is crucial for regulation of chromatin remodeling, DNA replication, repair, recombination, methylation, and transcription (Lee et al., 2000). The molecular function of HELLS is to facilitate *de novo* DNA methylation, which appears to play an important role in embryonic stem cell differentiation by silencing stemness genes (Xi et al., 2009). However, functional roles of the protein in tumor initiation and metastasis development are unknown. Higher levels of circulating *HELLS* mRNA may be a reflection of higher numbers of circulating tumor cells in metastatic patient blood, suggesting that HELLS may regulate tumor cell migration and invasion, as metastasis-competent cells are most likely invasive and migratory, enabling access to a tumor vascular system. Although HELLS mRNA expression levels are very low in normal peripheral blood lymphocytes, they increase significantly when the cells are activated and stimulated to proliferate (Lee et al., 2000). This implies that the status of immunologic responses of patients may affect outcome, and this should be carefully considered in future prospective validation studies.

Quantitative detection of cell-free CNAs in patient blood may be a desirable approach to developing diagnostic, prognostic, and therapy response-predicting markers (Swarup and Rajeswari, 2007). Since the seminal study demonstrating the feasibility of RT-PCR for detection of melanoma in peripheral blood (Smith et al., 1991), several reports have suggested that RT-PCR detection of circulating melanoma cells may have prognostic significance. The majority of these studies used RT-PCR detection of melanocytic differentiation antigens including tyrosinase, MART-1, and MAGE3 gene transcripts (Quaglino et al., 2004). Notably, Scoggins et al. (2006) reported that RT-PCR detection of tyrosinase and other markers in peripheral blood showed significant differences in prognoses (Scoggins et al., 2006). In this paper, we analyzed a novel class of genes that appear to be functionally associated with the development of melanoma metastasis and demonstrated that quantitative measurement of these gene transcripts may have potential value as a prognostic marker for melanoma metastasis. Given the functional association of this new class of candidate genes with tumor cell proliferation and metastasis, rather than melanin biosynthesis, RT-PCR detection of these novel markers can be used for assessment of prognostic risk in patients with amelanotic melanomas as well.

In conclusion, circulating mRNA levels of HELLS and NCAPH transcripts in melanoma patient blood were found to be significantly higher in patients with distant organ metastasis than in those with localized tumors. The area under ROC curves of *HELLS* and *NCAPH* were greater compared to serum LDH, which at present is one of the most useful serum prognostic indicators of metastatic melanoma. Multiple logistic regression analysis identified *HELLS* as a statistically significant independent marker of the presence of distant metastasis. This study shows that elevated serum levels of *HELLS* gene transcripts may have a better potential as serum biomarkers than LDH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Authors thank Dr. Wolfram Samlowski for comments on the manuscript. This work was supported by NIH K01 CA113779 and Nevada Cancer Institute (NVCi) Startup Grant for Dr. Ryu and DOE DEFG02-08ER64608 grant for Bio-repository program at NVCi.

References

- Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J. Clin. Oncol.* 2009; 27:6199–6206. [PubMed: 19917835]
- Becker D, Mihm MC, Hewitt SM, Sondak VK, Fountain JW, Thurin M. Markers and tissue resources for melanoma: meeting report. *Cancer Res.* 2006; 66:10652–10657. [PubMed: 17108101]
- Cook MG, Spatz A, Bocker EB, Ruiter DJ. Identification of histological features associated with metastatic potential in thin (<1.0 mm) cutaneous melanoma with metastases. A study on behalf of the EORTC Melanoma Group. *J. Pathol.* 2002; 197:188–193. [PubMed: 12015742]
- Deichmann M, Benner A, Bock M, Jackel A, Uhl K, Waldmann V, Naher H. S100-Beta, melanoma-inhibiting activity, and lactate dehydrogenase discriminate progressive from nonprogressive American Joint Committee on cancer stage IV melanoma. *J. Clin. Oncol.* 1999; 17:1891–1896. [PubMed: 10561230]
- Lee DW, Zhang K, Ning ZQ, Raabe EH, Tintner S, Wieland R, Wilkins BJ, Kim JM, Blough RI, Arceci RJ. Proliferation-associated SNF2-like gene (PASG): a SNF2 family member altered in leukemia. *Cancer Res.* 2000; 60:3612–3622. [PubMed: 10910076]
- Quaglino P, Savoia P, Osella-Abate S, Bernengo MG. RT-PCR tyrosinase expression in the peripheral blood of melanoma patients. *Expert Rev. Mol. Diagn.* 2004; 4:727–741. [PubMed: 15347265]
- Ryu B, Kim DS, Deluca AM, Alani RM. Comprehensive expression profiling of tumor cell lines identifies molecular signatures of melanoma progression. *PLoS ONE.* 2007; 2:e594. [PubMed: 17611626]
- Scoggins CR, Ross MI, Reintgen DS, et al. Prospective multi-institutional study of reverse transcriptase polymerase chain reaction for molecular staging of melanoma. *J. Clin. Oncol.* 2006; 24:2849–2857. [PubMed: 16782924]
- Smith B, Selby P, Southgate J, Pittman K, Bradley C, Blair GE. Detection of melanoma cells in peripheral blood by means of reverse transcriptase and polymerase chain reaction. *Lancet.* 1991; 338:1227–1229. [PubMed: 1719320]
- Swarup V, Rajeswari MR. Circulating (cell-free) nucleic acids – a promising, non-invasive tool for early detection of several human diseases. *FEBS Lett.* 2007; 581:795–799. [PubMed: 17289032]
- Winnepenninckx V, Lazar V, Michiels S, et al. Gene expression profiling of primary cutaneous melanoma and clinical outcome. *J. Natl Cancer Inst.* 2006; 98:472–482. [PubMed: 16595783]
- Xi S, Geiman TM, Briones V, Guang Tao Y, Xu H, Muegge K. Lsh participates in DNA methylation and silencing of stem cell genes. *Stem Cells.* 2009; 27:2691–2702. [PubMed: 19650037]

Figure 1.

Distribution patterns of circulating gene transcripts in patient sera and area under the receiver operating characteristic (AUC-ROC) curves. (A) Comparison of quantitative mRNA copy number for the genes *HELLS*, *NCAPH*, *TYMS*, *BIRC5*, and *ACTB* in patient sera among the sample groups (N: normal control, P: patients with Stage 0/1 primary melanomas, M: patients with Stage IV metastatic melanomas). *ACTB* served as a negative control. (B) Comparison of serum LDH concentrations in the patients with primary and metastatic melanomas. (C) AUC-ROC curves for each individual analyte are shown. Analyte *HELLS* shows the highest value.

Table 1

Logistic regression predicting probability of each gene transcript for the presence of metastatic melanomas

Biomarkers (candidate genes)							
Statistic	LDH	HELLS	NCAPH	TYMS	BIRC5	ACTB	
Logistic Regression P-value	0.203	0.005 ^b	0.016 ^b	0.108 ^b	0.999 ^b	0.939	
Odds Ratio ^a	NR	94.2	10.5	NR	NR	NR	

^a Odds ratios associated with log10 increase reported for each statistically significant biomarker.

^b Bonferroni adjusted p-values for evaluating four test genes.