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Original contribution

# Gene expression screening identifies CDCA5 as a potential therapeutic target in acral melanoma☆,☆☆

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**Summary** Acral melanoma (AM) is a rapidly progressing subtype of melanoma with poor prognosis. The complete array of molecular changes that occur during AM metastasis remains unclear. In this study, we compared the gene expression profiles of 6 primary and 12 lymph node metastatic AM samples by tissue microarray analysis. We found that the expression levels of 396 genes were increased and that of 766 genes were decreased in the metastatic tissues compared with that in the primary tumors. The top 19 genes upregulated in the metastatic tissue specimens were selected for high-content short interfering RNA screening. We found that inhibition of cell division cycle–associated 5 (CDCA5) significantly suppressed AM cell migration and invasion. Furthermore, we demonstrated that upregulation of CDCA5 was correlated with higher tumor-node-metastases stages ($P$ = .025) and a shorter disease-free survival in patients with AM ($P$ = .038). Cox regression analyses showed that high CDCA5 expression was also an independent factor of disease-free survival for patients with AM (hazard ratio =1.86, $P$ = .041). Overall, our data define the gene expression signature of AM metastasis and indicate that CDCA5 is a potential therapeutic target in AM.


## 1. Introduction

Melanoma is a malignancy that progresses rapidly and has poor prognosis [1,2]. Distant metastasis, which often occurs prior to diagnosis [3], is the major cause of mortality. Acral melanoma (AM), a subtype of melanoma, occurs on the palms, soles, and even nail beds and is associated with extensive metastasis and worse prognosis than common cutaneous melanoma [4]. The development and progression of AM are multistep processes accompanied by molecular changes.

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Various studies have revealed gene mutations in AM, including mutations in *BRAF*, *NRAS*, and *CKIT* [4,5]. Clinical studies have indicated that molecular-targeted therapy is the primary strategy for the treatment of metastatic melanoma. However, the frequency of these mutations in AM is much lower than in cutaneous melanoma [5-7]; therefore, no genotype-specific treatments are available for most patients with metastatic AM. In addition, the dysregulated genes involved in the metastatic cascade of AM remain unidentified, and the functions of specific genes have not been evaluated in AM cells.

Tumor metastasis is a complex process involving numerous steps and factors that contribute to accelerate the progress of metastasis from primary tumors [8]. Lymph nodes act as barriers to prevent the spread of tumor cells through the lymphatic tract, which is the first step in distant metastasis in most patients. Lymph node status is therefore an important prognostic factor in melanoma, and lymph node biopsy is used as a basis for judging clinical stages [9]. A multicenter clinical trial demonstrated that patients with a positive sentinel lymph node had shorter 5-year disease-free survival (DFS) compared with patients with negative sentinel lymph node [10]. Elucidation of the molecular events underlying lymph node metastasis can undoubtedly help deepen our understanding of mechanisms involved in metastatic AM and help identify more effective therapeutic targets.

Primary and metastatic tumors have the same cellular origin; it is therefore difficult to identify genomic markers that distinguish them. Microarray gene expression analysis has the advantages of being a large-scale, high-throughput, and highly efficient method that enables effective screening for differentially expressed genes and identification of potential drug targets.

In this study, we investigated the gene expression profiles of AM lymph node metastatic tissue samples via mRNA microarray analysis. Furthermore, the most significantly upregulated genes in metastatic AM samples were selected for functional validation via high-content siRNA screening. The results indicated that cell division cycle–associated 5 (CDCA5) is upregulated in AM metastatic tissues and that it primes AM cell migration. We additionally demonstrated that CDCA5 expression is correlated with AM progression and the DFS. Overall, our findings provide insights into the molecular basis of AM metastasis and suggest that CDCA5 serves as a therapeutic target for AM.

## 2. Materials and methods

### 2.1. Patients and specimens

Five patients who underwent surgery for primary AM, 11 for lymph node metastasis, and 1 for primary and matched lymph node metastasis were included in the microarray gene expression analysis. Tissue specimens were immediately snap-frozen and stored at −80°C. A second group of paraffin-embedded primary AM samples from 63 patients and metastatic AM samples from 46 patients (including 39 lymph nodes and 7 lung metastases) was collected for quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and immunohistochemistry (IHC). Samples from the 2 patient cohorts were separately submitted to the Department of Pathology at Peking Cancer Hospital and Institute between October 2013 to April 2016 for routine diagnosis, which was performed by experienced clinical pathologists based on hematoxylin and eosin staining and IHC analysis. The study protocol was approved by the Medical Ethics Committee of the Peking University Cancer Hospital and Institute.

### 2.2. Cells and cell culture

The A875 cell line was obtained from the American Type Culture Collection, and the AM cell line-2 (AMC-2 cell line) was derived from a hospitalized patient with AM, as previously described [11]. Cells were cultured in Dulbecco modified Eagle medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), penicillin (100 U/mL), streptomycin (100 $\mu$g/mL; Invitrogen), and L-glutamine (3 mmol/L) at 37°C in an atmosphere of 5% $CO_2$ and 95% air. The cells were passaged 2 or 3 times per week.

### 2.3. Analysis of mRNA expression using the GeneChip array

Total RNA was isolated from AM tissues and analyzed using the GeneChip PrimeView Human Gene Expression Array (Affymetrix, Santa Clara, CA). RNA quality was evaluated using a 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) as previously described [12]. The array cartridge allowed expression profiling using probe sets with established, well-annotated content; the sequences used in the array were selected from RefSeq v.36 and UniGene database 219 and included full-length human mRNAs from GenBank. Genes showing differential expression (fold change >2, $P < .05$) in metastatic AM relative to primary tumor samples were used for subsequent experiments.

### 2.4. High-content short interfering RNA screening

Combined RNA interference mixed viruses were constructed for functional screening. In brief, the short interfering (si)RNA sequences targeting 19 genes (Supplementary Table S1) and enhanced green fluorescent protein fusion genes were inserted into the GV248 lentiviral expression vector (GeneChem, Shanghai, China). Lentiviruses were mixed with Polybrene (5 mg/mL) and added to A875 cells. After cellular fusion, a scratch was made on the bottom of the culture plate with a 100-$\mu$L sterile pipette tip. Six images for each well were acquired at ×4 magnification after 0, 8, and 24 hours. The open wound area (relative to 0-hour values) was quantified using ImageJ software (National Institutes of Health, Bethesda, MD). The migration inhibition ratio was calculated as fold change =