Functional Prediction of Long Noncoding RNAs in Cutaneous Melanoma Using a Systems Biology Approach

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ABSTRACT: Cutaneous melanoma is the most aggressive type of skin cancer which its incidence has significantly increased in recent years worldwide. Thus, more investigations are required to identify the underlying mechanisms of melanoma malignant transformation and metastasis. In this context, long noncoding RNAs (lncRNAs) are a new type of noncoding transcripts that their dysregulations are associated with almost all cancers including melanoma. However, the precise functional roles of most of the significantly altered lncRNAs in melanoma have not yet been fully inspected. In this study, a comprehensive list of lncRNAs was interrogated across cutaneous melanoma samples to identify the significantly altered/dysregulated lncRNAs. To this end, lncRNAs were filtered in several steps and the selected lncRNAs projected to a bioinformatic and systems biology analysis using several publicly available databases and tools such as GEPIA and cBioPortal. According to our results, 30 lncRNAs were notably altered/dysregulated in cutaneous melanoma most of which were co-expressed with each other. Also, co-expression/alteration and differential expression analyses led to the selection of 12 out of these 30 lncRNAs as cutaneous melanoma key lncRNAs. Furthermore, functional demonstrated that these 12 lncRNAs might be involved in melanoma-relevant biological processes and pathways. In addition, the end result of our analyses demonstrated that these lncRNAs are associated with the clinicopathological features of melanoma patients. These 12 lncRNAs need to be further investigated in future studies to characterize their exact roles in melanoma development and to identify their potential for being used as drug targets and/or biomarkers for cutaneous melanoma.

KEYWORDS: Cutaneous melanoma, long noncoding RNAs (lncRNAs), bioinformatics, systems biology

Introduction

Melanoma is the most lethal type of skin cancer whose prevalence has significantly increased during the last decades.[1] Among all invasive melanoma cases, almost 5% are associated with inheritance and familial setting, which indicates that low-penetrance/high-prevalence genes do not have much of a role in the incidence of melanoma rate in the world. However, environmental factors, especially sun exposure, play a significant role in melanoma initiation and progression.[2] Although sun exposure is the major risk factor for cutaneous melanoma, specific genetic alterations are also associated with melanoma risk.[3] Cutaneous melanoma is hard to treat, and the available drugs and recent advancements in different treatment strategies including targeted therapy have not significantly increased the survival rate and period of melanoma patients.[4,5] However, recent advancements in melanoma immunotherapy have been considerably promising.[6,7]

Long noncoding RNAs (lncRNAs) are a new type of non-protein coding transcripts that are present in almost all organisms and have unique features that differentiate them from messenger RNAs (mRNAs).[8] These long transcripts are usually more than 200 nucleotides, do not code for protein, and are involved in a variety of regulatory mechanisms including gene expression.[9] Actually, lncRNAs play their roles, in relation to other molecules, as scaffolds, signals, guides, and decoys.[10]

Cumulative evidence are reporting the association of lncRNAs with developmental processes and different diseases, but the precise mechanism of action of most of them is not still elucidated.[11] Also, advancements in high-throughput sequencing technologies have led to the discovery of an increasing number of novel transcripts including lncRNAs, most of which are not still fully annotated.[12]

LncRNAs play key roles in a variety of diseases including cancer. In fact, they involve in the epigenetic regulation of other macromolecules through chromatin remodeling, as well as transcriptional and post-transcriptional regulation, and, consequently, their dysregulations lead to different diseases such as cancer.[13] In other words, lncRNAs interact with other biological macromolecules including DNA, protein, and RNA and mediate several cancer manifestations.[14] Frequent studies are reporting that a significant proportion of the genomic mutations in cancer occur within nonprotein coding regions, usually inside the lncRNA loci. Also, the association between aberrant expression of thousands of lncRNAs with different cancers has been demonstrated. However, only a small proportion of these lncRNAs have been functionally annotated. Notably, it has been indicated that these annotated lncRNAs involve in several cellular processes including proliferation, migration, or genomic stability and consequently their dysregulations drive malignant transformation.[15,16]

Since lncRNAs have a higher tissue specificity than mRNAs, they might adopt a dual functionality in cancer, executing either as an oncogene or tumor suppressor, according to their tissue of origin.[17] As stated earlier, the association between thousands of lncRNAs and cancer has been reported. Among all, HOTAIR is the most known lncRNA in the context of cancer which plays an oncogenic role in many of the human malignancies including melanoma.[9] Several other lncRNAs such as BANCR, MALAT1, UCA1, ANRIL, and SNHG5 are also connected with cutaneous melanoma and were reviewed by Hulstaert et al,[5] some of which could be considered as biomarkers or therapeutic targets for malignant melanoma.[5] Also, several studies have reported the association of individual lncRNAs such as LINC00673,[18] GAS5,[19] UCA1,[20] MEG3,[21] and PVT1[22] with melanoma. Even though, there are quite a number of lncRNAs that are significantly altered in cutaneous melanoma at the genomic and/or transcriptomic levels but are not still functionally annotated. In a similar study by Wang et al[1] on the association of lncRNAs with the tumorigenesis and metastasis of cutaneous melanoma, 295 lncRNAs were identified as DE-lncRNAs between normal skin and metastatic melanoma tumor of which 4 lncRNAs were common with our results. However, we could not find common results between our study and another similar study on the association of lncRNAs and melanoma done by Zhang et al.[23]

Bioinformatics and systems biology, in its simplest description, is the development and application of different computational methods and tools to convert biological "omic" data into the information that their interpretation could enhance the personalized treatment of patients.[24,25] In this study, we adopted an integrative bioinformatic and systems biology approach to examine if any of the known lncRNAs have significant associations with cutaneous melanoma. To this purpose, the processed data of TCGA Skin Cutaneous Melanoma study were interrogated. We also investigated the biological processes and pathways these lncRNAs might participate in the context of cutaneous melanoma. Overall, several novel candidate biomarkers and drug targets for cutaneous melanoma were presented in this study.

Materials and Methods

Data preparation

First, 3823 lncRNAs were retrieved from HGNC BioMart (https://biomart.genenames.org/)[26] (Supplementary file 1) according to the following criteria: HGNC Mart Genes Datasets; Filter by genes with RefSeqs accession; Status: Approved; Locus group: noncoding RNA; Locus type: RNA, long noncoding. Then, using cBioPortal database,[27,28] all lncRNAs were queried at both genomic and transcriptomic levels against the Skin Cutaneous Melanoma (TCGA, Provisional) study, comprising 478 samples, and lncRNAs with alterations (copy-number alteration [CNA] and expression dysregulation) in ≥10% of samples were selected for downstream analyses (30 genes; Supplementary file 2). cBioPortal is a database that facilitate in visualizing, analyzing, and downloading of large-scale cancer genomics data sets. Finally, the Expression (RNA Seq V2 RSEM), Expression z-Scores (RNA Seq V2 RSEM), and relative linear copy-number values of all selected lncRNAs were imported from the cBioPortal database into *R* statistical software by means of CDGS-R package v1.2.8 (https://CRAN.R-project.org/package=cgdsr). These data have been preprocessed and normalized by the cBioPortal database. The CGDS-R is a package for *R* statistical software that provides a basic set of functions for querying the Cancer Genomic Data Server (CGDS).

Genotranscriptomic analysis

The mean expression level of each lncRNA across all samples was calculated to compare the expression of these lncRNAs in cutaneous melanoma. Also, all 30 lncRNAs were interrogated across single-cell RNA-Seq data sets using scRNASeqDB (https://bioinfo.uth.edu/scrnaseqdb/index.php) and the expression of lncRNAs with significant rank in melanoma data sets were retrieved. It is also worth mentioning that the expression of our under-investigation lncRNAs were tested in scRNASeqDB regardless of the library preparation method used for the generation of different data sets. These analyses help identify the lncRNAs with noticeable expression in cutaneous melanoma at both bulk and single-cell levels. Furthermore, the correlation between CNA and z-Score expression data of each lncRNA was analyzed using the Spearman method to identify the lncRNAs which their dysregulation could have been due to their genomic alterations.

Analysis of the co-alteration/expression of lncRNAs with each other

The significant mutual exclusivity and co-occurrence of alterations among 30 lncRNAs were obtained from cBioPortal to interrogate the interactions of these lncRNAs (Supplementary file 3). Then, gene pairs with significant co-occurrent alterations were recruited to reconstruct an association network using Cytoscape v3.5.1 (http://www.cytoscape.org).[29] Also, to identify the hub (most important) lncRNAs within the association network, the topology of the network was analyzed using the Analyzer tool of the Cytoscape software. Similarly, both expression and z-Score expression data were used to analyze the coexpression and z-Score coexpression (i.e. the correlation between z-Score expression data) of 30 lncRNAs, respectively. To this purpose, the correlation of both expression and z-Score expression data was calculated independently using the Pearson method, visualized as an integrated network and analyzed using Cytoscape v3.5.1.

Differential expression analysis

GEPIA web server (http://gepia.cancer-pku.cn)[30] was used to investigate the differential expression of 30 lncRNAs in

cutaneous melanoma according to the TCGA RNA-Seq data and using the LIMMA method. GEPIA is a web server that interactively analyzes the RNA-Seq expression data of tumor and normal samples from the TCGA and the GTEx projects. Furthermore, a comprehensive literature review was conducted to find if the same dysregulations of these lncRNAs have been previously reported in melanoma or other cancers.

Selection of key lncRNAs

To focus our investigations on the most important lncRNAs, we combined the list of cutaneous melanoma differentially expressed lncRNAs (DE-lncRNAs) with the hub lncRNAs derived from the topological analysis of co-alteration/expression networks and named them as the "cutaneous melanoma key lncRNAs" (CMK-lncRNAs). Accordingly, the CMK-lncRNAs include 2 groups of lncRNAs: (1) those lncRNAs that are both significantly differentially expressed in cutaneous melanoma samples compared with normal ones and have genomic alterations in over 10% of cutaneous melanoma samples; (2) those lncRNAs that are both hubs of the cutaneous melanoma co-alteration/expression networks and have mutations/CNAs (genomic alterations) in over 10% of cutaneous melanoma samples. Also, all downstream analyses were implemented based on the CMK-lncRNAs.

Coexpression analysis of CMK–lncRNAs with other genes

The coexpression of CMK-lncRNAs with each other and all other genes were interrogated to predict the functional similarity of these lncRNAs according to their associated genes (guilt by association principle). To obtain the coexpressed genes with the highest level of confidence, the preprocessed data of both cBioPortal and GEPIA databases were integrated. To this end, the coexpression of each CMK-lncRNA with other genes in the skin cutaneous melanoma (TCGA, Provisional) study was analyzed independently using cBioPortal, and genes with Pearson and Spearman correlation coefficient over 0.3 were outputted (Supplementary file 4). Besides, the coexpression of CMK-lncRNAs with other genes in the cutaneous melanoma TCGA study was evaluated via GEPIA web server, and gene pairs with Pearson correlation coefficient (PCC) over 0.3 were selected (Supplementary file 5). Then, the intersection of coexpression data derived from cBioPortal and GEPIA databases was identified. Moreover, to increase the sensitivity of our downstream analyses, the expression status (either dysregulation or normal expression) of coexpressed genes was imposed to that of CMK-lncRNAs and the inconsistencies were filtered out. To this purpose, the list of all differentially expressed genes in cutaneous melanoma TCGA study was retrieved from the GEPIA web server (Supplementary file 6) and subjected to the expression data; if the CMK-lncRNA was dysregulated, the coexpressed genes that were dysregulated were retained, and if

the CMK-lncRNA was normally expressed, the coexpressed genes with normal expression were selected.

Functional prediction

The coexpression data obtained in the previous step were used to perform a computational functional analysis. First, the coexpressed genes of CMK-lncRNAs were employed to perform a gene set enrichment analysis of gene ontology-biological process (GO-BP) terms using Enrichr web server (http://amp. pharm.mssm.edu/Enrichr).[31,32] Next, all of the CMK-lncRNAs were interrogated via FuncPred database (http://www.funcpred. com)[33] to investigate the association of them with GO-BP terms in normal skin tissue based on the evolutionary conserved and tissue-specific expression data. Finally, first-ranked GO-BP terms of FuncPred (according to the lowest FDR) and Enrichr (according to the highest combined score) results as well as their intersection were selected as the most significant GO-BP terms. Finally, the GO-BP terms and their associated lncRNAs were used to reconstruct an association network and identify the most important CMK-lncRNAs within that using the Cytoscape. Besides, considering the modulated lncRNAs in the lncRNA-GO-BP network, the ConsensusPathDB database (http://cpdb.molgen.mpg.de)[34,35] was used to figure out if coexpressed genes of CMK-lncRNAs in each module are overrepresented in any of the Reactome pathways. Also, a literature review was performed to investigate the association between identified biological processes and first-ranked pathways with cutaneous melanoma development.

Clinicopathological analysis

At last, the association between the CMK-lncRNAs and the clinicopathological features of cutaneous melanoma patients was assessed. To this end, the GEPIA web server was used to analyze the prognostic value of all CMK-lncRNAs using the Kaplan–Meier estimates. Likewise, the differential expression of CMK-lncRNAs between different stages of cutaneous melanoma was investigated using the GEPIA web server. In both of these analyses, *R* statistical software was also used to perform a multiple testing correction and calculate the adjusted *P* values (*p.adj*) using the "Hochberg" method. Moreover, the CMK-lncRNAs that were differentially expressed (Table 1) were focused to further investigate the clinicopathological significance of their dysregulations in cutaneous melanoma. To this purpose, the samples containing significant alterations in the expression ($|$expression z-Score$| > 2$) of any of the 9 focused CMK-lncRNAs were extracted from the skin cutaneous melanoma (TCGA, Provisional) data set. Then, the clinicopathological data of extracted samples were imported from the cBioPortal database into *R* statistical software by means of CDGS-R package v1.2.8. Finally, the overall survival (OS), disease-free survival (DFS), and pathological tumor stage of all extracted samples were studied against the selected genes.

Table 1. DE-lncRNAs in cutaneous melanoma.

GENE SYMBOL	GENE SYMBOL	DYSREGULATION	LOG2FC	ADJ P*	PREVIOUS CONFIRMATION
LINC00518	long intergenic nonprotein coding RNA 518	Over-expressed	4.03	2.22E–257	Melanoma[36]
LINC00174	long intergenic nonprotein coding RNA 174	Under-expressed	–1.404	9.00E–185	Glioma[37]
LINC00467	long intergenic nonprotein coding RNA 467	Over-expressed	1.77	1.78E–224	Lung adenocarcinoma[38]
CASC15	cancer susceptibility 15	Over-expressed	1.674	5.88E–151	Melanoma[39]
LINC00964	long intergenic nonprotein coding RNA 964	Under-expressed	–1.521	5.27E–215	Esophageal cancer[21] and HNSCC**[40]
LINC00265	long intergenic nonprotein coding RNA 265	Under-expressed	–1.08	1.65E–121	Colorectal Cancer[41]
MIR205HG	MIR205 host gene	Under-expressed	–7.482	0.00E+00	Cervical cancer[42]
HCP5	HLA complex P5	Under-expressed	–1.219	4.76E–47	Melanoma[43]
LINC00662	long intergenic nonprotein coding RNA 662	Over-expressed	1.018	6.55E–134	Lung cancer[44]

*Adjusted *P* value.
**Head and neck squamous cell carcinoma.

Statistical and topological analysis

All the statistical analyses were done by *R* statistical software, *R* Development Core Team (2014), freely available at http://www.r-project.org. The absolute *z*-Score > 2 was considered as the significant expression dysregulation throughout the study. Also, the absolute Pearson and Spearman correlation coefficients >0.3 were considered as statistically significant. Moreover, the *P*-value and *P adj* < .05 was used as the significance threshold in all of the analyses. Regarding the topological analysis of networks, 2 different network metrics including degree (the number of edges incident to each node) and betweenness centrality (a measure of node centrality based on shortest paths) were simultaneously employed to identify the hub nodes.

Results

Remarkable expression of lncRNAs in cutaneous melanoma

The genotranscriptomic analysis of all lncRNAs using cBio-Portal demonstrated that 30 lncRNAs had significant alterations (CNA and expression dysregulation) in ≥10% of samples (Figure 1). Also, it is notable that 29 out of 30 lncR-NAs had up-regulative (i.e. amplification and over-expression) alterations, while CDKN2A-AS1 lncRNA was mostly deleted in cutaneous melanoma. The expression data of 30 lncRNAs, retrieved from cBioPortal, demonstrated that all of them except LINC00051 and HPYR1 have notable expression in cutaneous melanoma (Figure 2A). Similarly, the single-cell RNA-seq analysis of lncRNAs demonstrated that 21 out of 30 lncRNAs were significantly expressed in a single-cell RNA-seq melanoma data set (GEO series GSE72056). Although, the analyzed data of only 19 lncR-NAs were accessible (Figure 2B). Interestingly, HPYR1

lncRNA had considerable expression at the single-cell level. Furthermore, analysis of the correlation between CNA and *z*-Score expression data of lncRNAs demonstrated that the *z*-Score expression of 19 out of 30 lncRNAs is significantly correlated (rho > 0.3, *P*-value < 1.91E–09) with their copy-number values (Figure 2C).

Co-alteration/expression of 30 lncRNAs

The network of gene pairs with co-occurrent alterations illustrated that the selected lncRNAs have notable co-occurrent alterations with each other (Figure 3A). According to the topological analysis of this network, the lncRNAs LINC00240, SNHG11, and HCG18 were identified as hub nodes of the network. Also, the coexpression analysis of lncRNAs indicated that 20 out of 30 lncRNAs are significantly coexpressed with each other (PCC > 0.3) according to both coexpression and *z*-Score coexpression data (Figure 3B). Likewise, the topological analysis of coexpression network revealed the lncRNAs LINC00265, SNHG11, and HCG18 as hub nodes of the network.

Differential expression of lncRNAs in cutaneous melanoma compared with normal samples

The differential expression analysis of 30 lncRNAs according to the TCGA and GTEx RNA-Seq data demonstrated that 9 out of 30 lncRNAs were differentially expressed (|Log2FC|> 1, *q* value < 0.05) in cutaneous melanoma 5 of which were under-expressed while others were over-expressed (Table 1). Interestingly, the literature review demonstrated same dysregulations of 5 out of these 9 lncRNAs in melanoma or other cancers. Among all, LINC00518 lncRNA had the most remarkable differential expression (Log2FC = 4.03) in TCGA cutaneous melanoma samples compared with normal ones.
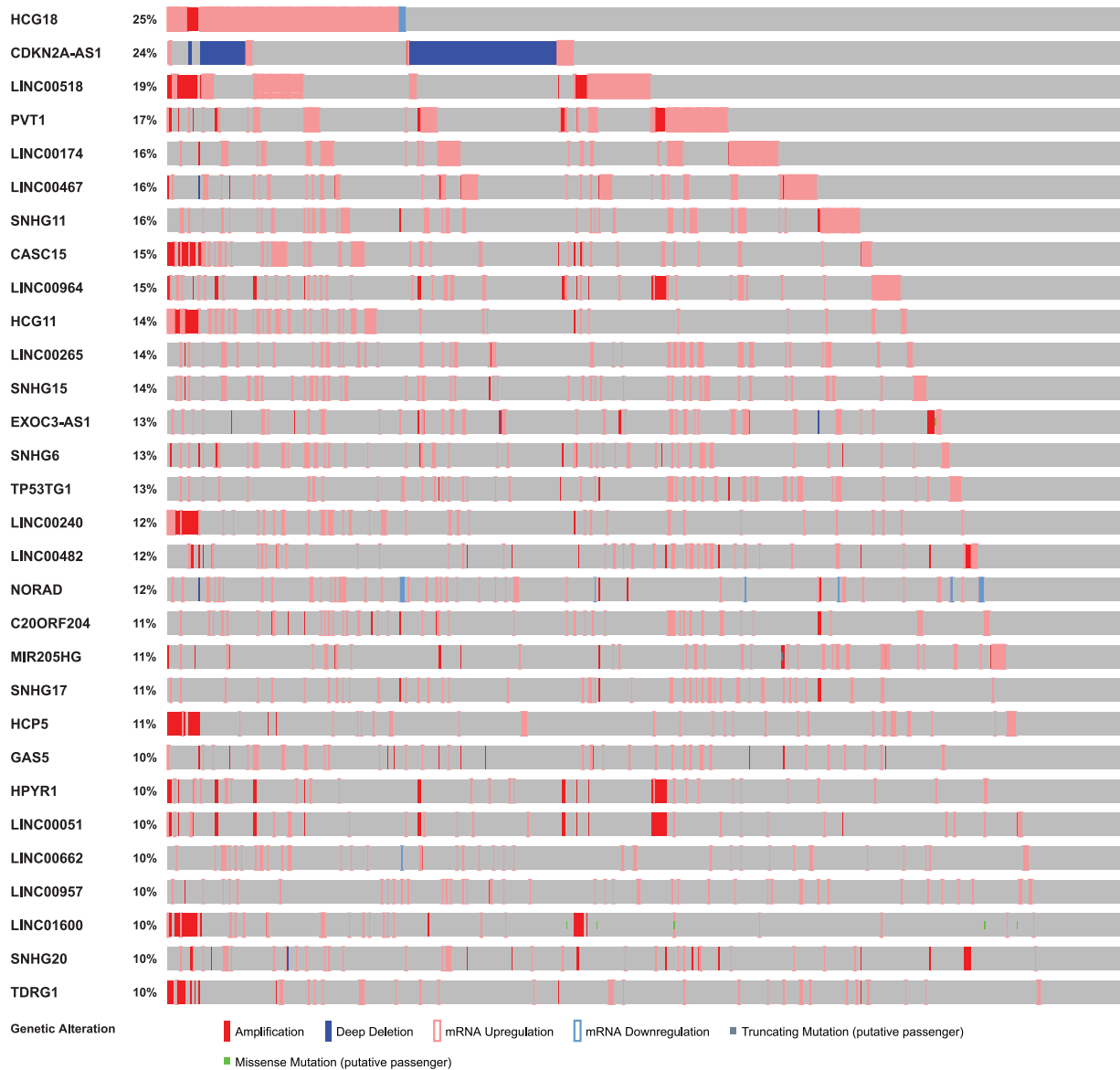
Figure 1. 30 lncRNAs with genotranscriptomic alterations in ≥10% of samples of TCGA skin cutaneous melanoma.
The percentages on the Y axis are indicative of the proportion of samples with genomic and transcriptomic alterations in the corresponding lncRNAs in cutaneous melanoma. Blue and red colors demonstrate the type of genomic or transcriptomic alterations, and the light gray color is indicative of samples with no alteration in the corresponding lncRNAs in TCGA skin cutaneous melanoma. This figure was achieved by means of cBioPortal database.

Functional roles of CMK-lncRNAs

Twelve out of 30 lncRNAs were finally selected as the CMK-lncRNAs. The coexpression analysis of CMK-lncRNAs with other genes using both cBioPortal and GEPIA web server demonstrated that they are significantly coexpressed (PCC > 0.3) with other genes (Supplementary file 7). The coexpressed genes with any of the CMK-lncRNAs were used to perform a gene set enrichment analysis, which demonstrated that most of the CMK-lncRNAs might be associated with cancer-related biological processes (Figure 4; Supplementary file 8). For instance, the potential of melatonin, as a potent anti-neoplastic factor, in the suppression of cell growth in some cutaneous and uveal melanoma cell lines have been previously demonstrated, which might be associated with the involvement of melatonin in the regulation of keratinocyte

proliferation (GO:0010837; associated with CASC15).[45] Also, it has been demonstrated that targeting of NGLY1, a protein deglycosylation (GO:0006517; associated with LINC00265) enzyme, could promote the efficiency of melanoma targeted therapy and chemotherapy by inducing stress signaling-associated apoptosis, pleiotropic responses, and cytokine surges.[46] Similarly, the genes involved in the Regulation of Macrophage Chemotaxis (GO:0010758; associated with LINC00174) are notably downregulated in FoxO1-deficient macrophages which results in the transition of macrophages to an M2-like/TAM-like phenotype and development of melanoma.[47] Moreover, it has been reported that genes involved in antigen receptor-mediated signaling pathway (GO:0050851; associated with LINC00265) could be used for predicting prognosis and immunotherapy response
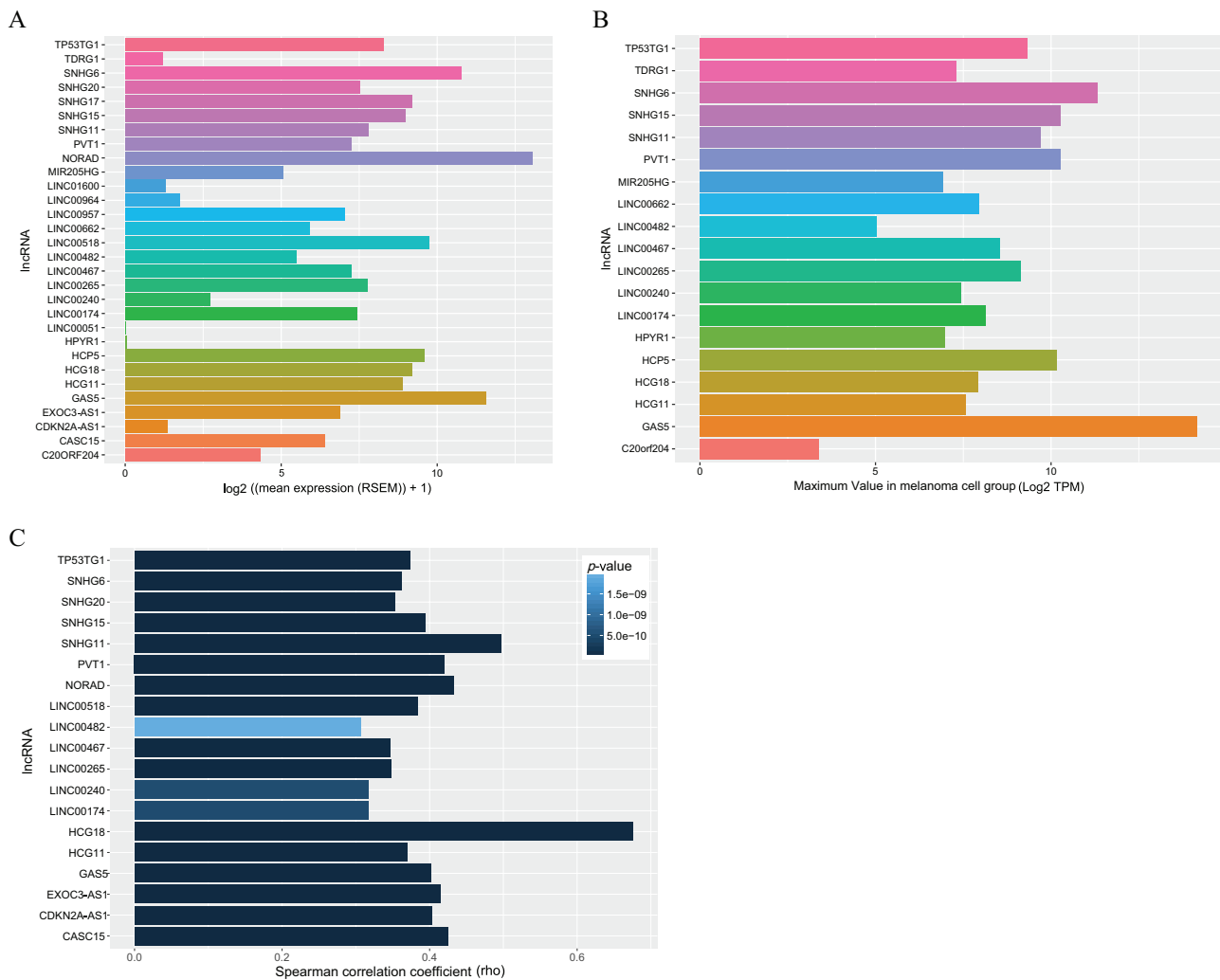
Figure 2. The expression of selected lncRNAs in cutaneous melanoma: (A) Mean expression levels of selected lncRNAs in cutaneous melanoma according to the data retrieved from cBioPortal. Each color represents a different lncRNA and the mean expression levels are log2 transformed. (B) maximum expression levels of selected lncRNAs in melanoma cell groups according to the data obtained from scRNASeqDB database. Each color represents a different lncRNA and the maximum expression values of lncRNAs in melanoma cell groups are $\log^2$ transformed. (C) The correlation between *z*-score expression and copy-number alteration data of selected lncRNAs in cutaneous melanoma. All of these figures were drawn by *R* statistical software.

in melanoma.[48] Furthermore, Zhang et al[49] showed that downregulated genes in uveal melanoma were involved in several pathways including regulation of Ras protein signal transduction (GO:0046578; associated with LINC00265 and LINC00174). Interestingly, several of these melanoma-associated biological processes were connected to lncRNAs of the module B of the lncRNA-GO-BP network (Figure 4). In addition, according to the pathway enrichment analysis via ConsensusPathDB database, coexpressed genes of CMK-lncRNAs in Module B of the lncRNA-GO-BP network were significantly enriched (*P*-value and *q* value < .05) in different Reactome pathways (Supplementary file 9) of which the "*Cytokine Signaling in Immune system*" pathway was identified as the first-ranked result (*P*-value = 2.37E–11, *q* value = 3.41E–09). Notably, 27 out of 217, 12.44%, of coexpressed genes of lncRNAs in Module B of the lncRNA-GO-BP network were enriched in this pathway. It was demonstrated that

the "Cytokine signaling in Immune system" was among the 4 pathways most impacted by Rosiglitazone (RGZ) drug, the application of which promotes the growth and development of melanoma via activating the paracrine signaling.[50] Also, Chen et al[51] demonstrated that immune check-point blockade therapies promote melanoma treatment via influencing several immune-associated pathways including the "Cytokine signaling in Immune system."

Clinicopathological significance of CMK–lncRNAs

The survival analysis of CMK-lncRNAs across all TCGA cutaneous melanoma samples indicated that the expression level of 4 out of 12 CMK-lncRNAs was significantly associated (Logrank test *P adj* < .05) with the OS of cutaneous melanoma patients (Figure 5A). More precisely, while the expression level of LINC00518 and MIR205HG was
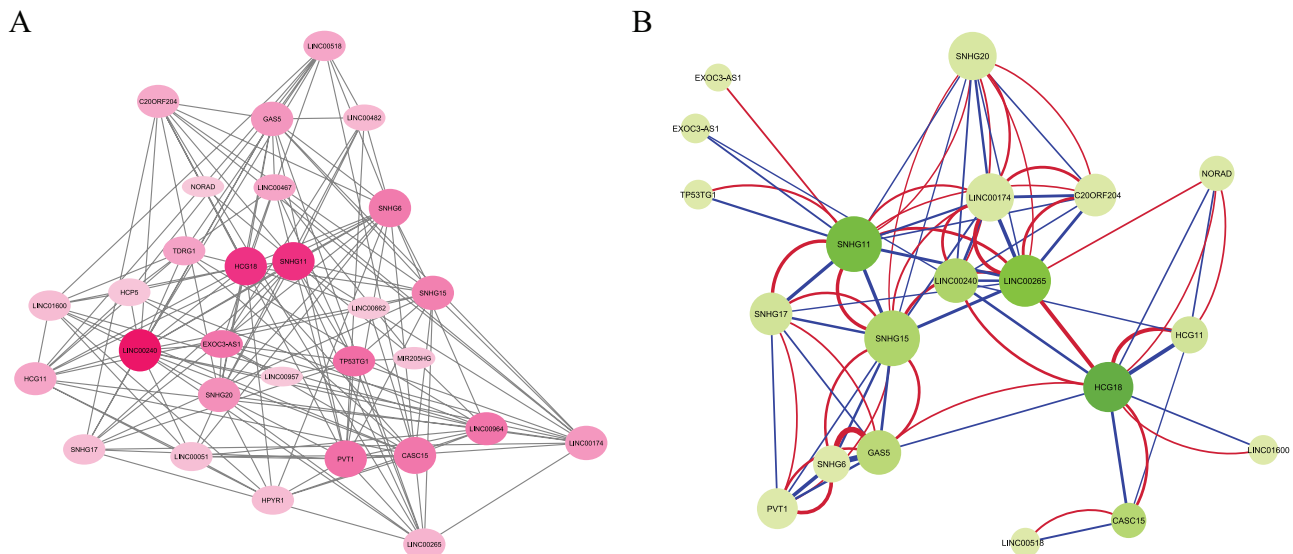
A

B

Figure 3. Co-expression/alteration of selected lncRNAs with each other: (A) co-occurrent alteration network of lncRNAs with each other in cutaneous melanoma. In this network, both genomic and transcriptomic alterations are considered. The intensity of purple node color is proportional to the betweenness centrality score; stronger purple color indicates higher betweenness centrality score. The node size is proportional to node degree; larger node indicates higher node degree. (B) co-expression network of lncRNAs with each other in cutaneous melanoma.
Red and blue edges are indicative of coexpression and z-Score coexpression of lncRNAs with each other, respectively. Also, the edge width demonstrates the Pearson correlation coefficient; wider edge is indicative of higher Pearson correlation coefficient. The intensity of green node color is proportional to the betweenness centrality score; stronger green color indicates higher betweenness centrality score. The node size is proportional to node degree; larger node indicates higher node degree.

negatively associated with the OS of cutaneous melanoma patients that of HCP5 and LINC00964 had positive correlation with the OS of patients. Similarly, the cutaneous melanoma pathological stage analysis of CMK-lncRNAs showed that the expression of 4 out of 12 CMK-lncRNAs is significantly altered (*P adj* < .05) in different stages of the cutaneous melanoma (Figure 5B). Moreover, the clinicopathological investigation of the cutaneous melanoma samples with dysregulation in any of the differentially expressed CMK-lncRNAs demonstrated that the OS and DFS of more than 56% of the selected samples were lower than 5 years, and more than 55% of the melanoma samples were in stage III or IV (Supplementary file 10).

Discussion

LncRNAs are a new type of noncoding transcripts that play pivotal regulatory roles in the cell. An increasing number of studies are reporting the association between lncRNA dysregulations and a variety of cancers. Cutaneous melanoma has not been an exception, and the involvement of a number of lncRNAs in the development of this malignancy has been previously demonstrated.[5] However, since melanoma is the most lethal type of skin cancer in the world, investigation of the lncRNAs involved in the initiation and progression of this cancer should be highly regarded. In this study, we passed 3823 lncRNAs through several filtration steps and conducted a bioinformatic and systems biology analysis to identify key lncRNAs associated with cutaneous melanoma and to characterize their functional roles. A similar study on the association of lncRNAs with survival of cutaneous melanoma patients[52] reported 6 significant lncRNAs but only

one of them, namely HCP5, was common with our results. In addition, while GEPIA and cBioPortal are well-known specialized cancer databases, significantly dysregulated lncRNAs obtained from these 2 databases did not include the previously reported melanoma-associated lncRNAs such as SPRY4-IT1 (SPRY4 intronic transcript 1).[53,54] This could possibly be referred to the differences between TCGA Research Network and individual/nonglobal research projects in the context of the cohort of melanoma patients/samples used and the adopted analysis protocol.

According to our results, 30 lncRNAs were significantly altered in cutaneous melanoma and, thus, could be essential factors in cutaneous melanoma initiation/progression. However, the differential expression analysis of these lncRNAs using the GEPIA web server demonstrated that 9 out of 30 lncRNAs were differentially expressed in cutaneous melanoma compared with normal samples. It is also worth mentioning that the dysregulation directionality (either up- or down-regulation) of only 4 out of 9 differentially expressed lncRNAs, including LINC00518, LINC00467, CASC15, and LINC00662, was consistent with the data retrieved from the cBioPortal. This is possibly due to the integration of TCGA SKCM normal (#1) and GTEx normal (#557) skin samples for differential analysis by the GEPIA web server, while cBioPortal database relies only on the TCGA data. Also, the positive correlation between expression dysregulations and CNA of 19 out of 30 lncRNAs demonstrated that amplifications/deletions of these lncRNAs play a key role in the alteration of their expression level. Moreover, the expression analysis also demonstrated that most of these lncRNAs had notable expression level according to
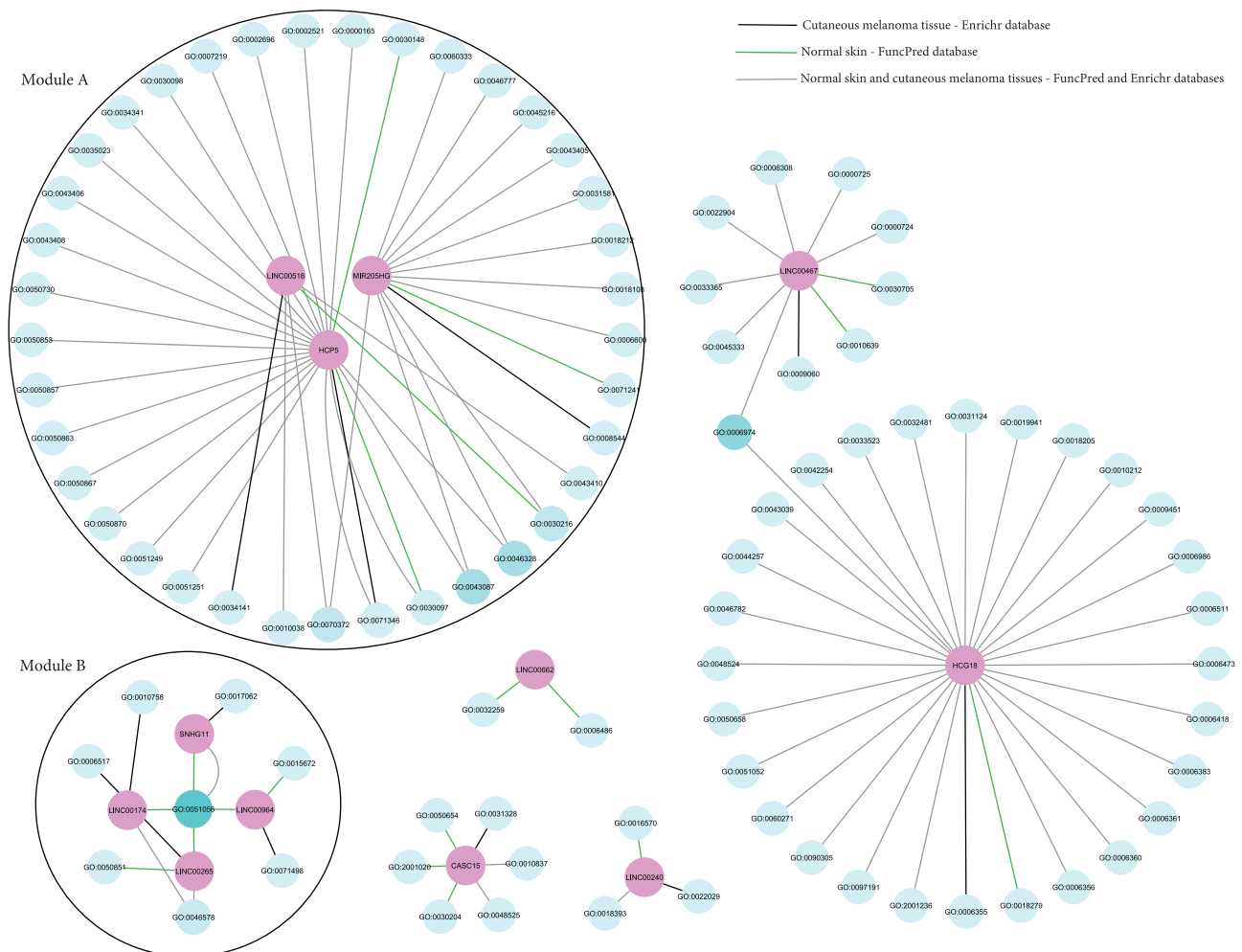
Figure 4. The lncRNA-GO-BP association network.
This network demonstrates the lncRNAs with different biological processes. Pink nodes represent CMK-lncRNAs. The intensity of blue node color indicates the betweenness centrality score; stronger blue color indicates higher betweenness centrality score. The edge color is representative of the source database of predicted GO term and the reference tissue type. The network was reconstructed using the Cytoscape software.

both single-cell and bulk RNA-seq data, which is confirming that these lncRNAs might play essential roles in cutaneous melanoma cells and possibly normal melanocytes.

The network analysis of co-occurrent alteration and coexpression of lncRNAs demonstrated that most of the selected lncRNAs are significantly coexpressed/co-altered in cutaneous melanoma. This indicates that these lncRNAs are possibly involved in common biological processes and pathways. In addition, topological analysis of these networks identified lncRNAs SNHG11, HCG18, LINC00265, and LINC00240 as the hub lncRNAs of which LINC00265 and LINC00240 have confirmed associations with lung adenocarcinoma[55] and esophageal squamous cell carcinoma,[56] respectively. However, there is still no evidence of the association of SNHG11 and HCG18 with neither melanoma nor other malignancies.

According to our applied methodology (see Methods), 12 lncRNAs, including, LINC00518, LINC00174, LINC00467, CASC15, LINC00964, LINC00265, MIR205HG, HCP5, LINC00662, HCG18, SNHG11, and LINC00240, were presented as CMK-lncRNAs. Although 3 out of 12 CMK-lncRNAs,

including HCG18, SNHG11, and LINC00240, were not differentially expressed according to the GEPIA web server, these lncRNAs might still be pivotal factors in cutaneous melanoma progression. Actually, these lncRNAs might be considered as "Class II cancer genes."[57] Class II cancer genes are those cancer-related genes that, though, are neither frequently mutated nor differentially expressed in cancer, they could play a role as "signal linkers" in coordinating cancerous signals between differentially expressed and mutated genes.[57]

The gene set enrichment analysis of coexpressed genes of CMK-lncRNAs indicated that most of the CMK-lncRNAs might be involved in cancer-associated biological processes. Among all, 3 biological processes including regulation of keratinocyte proliferation (GO:0010837), keratinocyte differentiation (GO:0030216), and epidermis development (GO:0008544) as well as immune-related biological pathways are the most remarkable ones in the context of cutaneous melanoma. Also, the pathway enrichment analysis of lncRNAs in Module B of the lncRNA-GO-BP network demonstrated that the coexpressed genes of these lncRNAs are most significantly
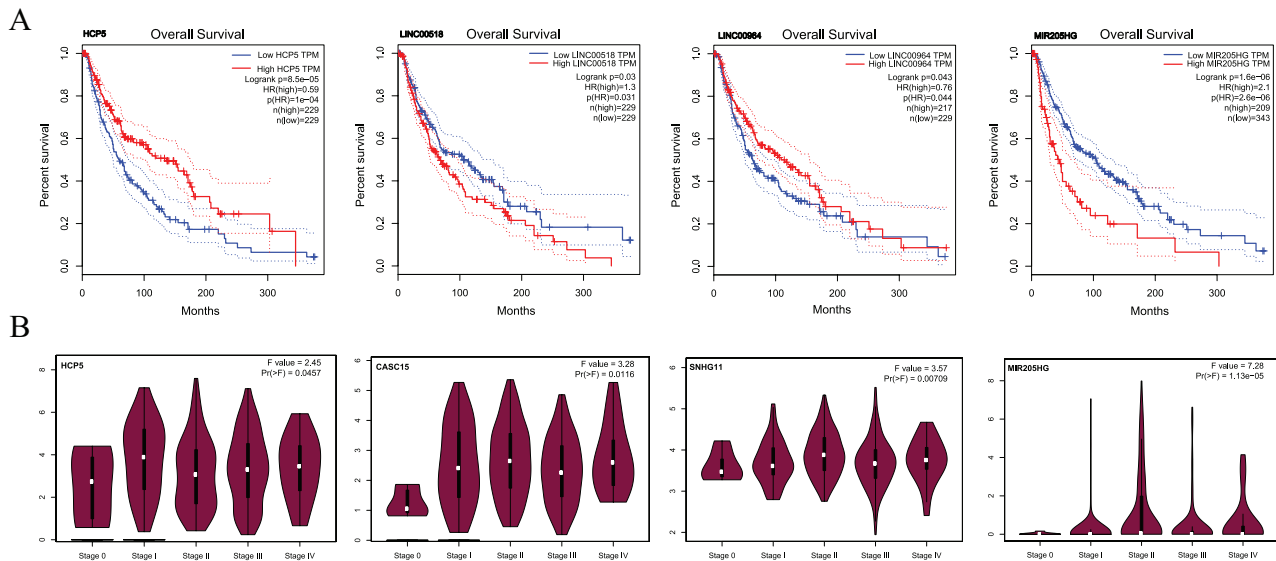
Figure 5. The prognostic value of CMK-lncRNAs for melanoma: (A) the expression level of 4 out of 12 CMK-lncRNAs had significant associations with survival rate of melanoma patients based on Logrank test analyses. (B) The significant differential expression of 4 out of 12 CMK-lncRNAs between different stages of cutaneous melanoma.
CMK-lncRNAs stands for cutaneous melanoma key lncRNAs. These plots were obtained using the GEPIA web server.

enriched in the "*Cytokine Signaling in Immune system*" pathway. This result indicates that CMK-lncRNAs in Module B might collaboratively/synergistically function in the same pathway. Fleming et al[58] previously demonstrated that miR-150 activity in the Cytokine Signaling in Immune system is associated with recurrence of melanoma. Also, the relation between "*Cytokine Signaling in Immune system*" pathway and 2 other cancer types including hepatocellular carcinoma[59] and prostate cancer[60] has been reported. Moreover, the "*Cytokine Signaling in Immune system*" pathway is associated with macrophage polarization and plasticity,[61] which is an important factor in the development of various malignancies including cutaneous melanoma.[62]

CMK-lncRNAs are also associated with clinicopathological features of cutaneous melanoma patients. According to the survival analyses, the expression level of 4 out of 12 CMK-lncRNAs, including HCP5, LINC00518, LINC00964, and MIR205HG, is associated with the OS of cutaneous melanoma patients. Thus, these lncRNAs could be collectively used for early prognosis and possibly diagnosis of cutaneous melanoma. In addition, LINC00518 and upstream negative regulators of 3 other lncRNAs could be considered as candidate drug targets for cutaneous melanoma patients. Similarly, the expression level of 4 out of 12 CMK-lncRNAs, including HCP5, CASC15, SNHG11, and MIR205HG, is associated with different stages of the cutaneous melanoma. Accordingly, the expression level of these lncRNAs could be considered for characterization of different stages of melanoma tumors. Furthermore, based on our results, most of the cutaneous melanoma samples with dysregulation in any of the differentially expressed CMK-lncRNAs belong to the stage III or IV melanoma patients with less than 5 years OS/DFS.

Conclusion

To sum up, this is a thorough study with the purpose of identifying most essential lncRNAs in the development of cutaneous melanoma. According to our results, among 3823 lncRNAs, 30 lncRNAs were proposed as the ones with most significant associations with cutaneous melanoma. Also, 12 out of these 30 lncRNAs were labeled as CMK-lncRNAs, and our downstream analyses demonstrated that most of these CMK-lncRNAs are associated with cancer-related biological processes. We also interrogated the functional roles of CMK-lncRNAs and demonstrated their associations with clinicopathological features of cutaneous melanoma patients. In short, the CMK-lncRNAs could be considered as candidate diagnostic/prognostic biomarkers and/or drug targets for cutaneous melanoma patients. Considering all of our results simultaneously, the lncRNA LINC00518 is the most remarkable one among all 12 CMK-lncRNAs and might be an essential oncogenic factor in the cutaneous melanoma development. However, further experimental assays are required to evaluate the potential of these lncRNAs in practice and to further characterize their precise roles in cutaneous melanoma tumorigenesis and metastasis.

Author Contributions

M.S. designed and performed bioinformatic and systems biology analyses and helped drafting the manuscript. Z.R. supervised the

whole study and revised the final version of the manuscript. All authors read and approved the final manuscript.

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Supplemental Material

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