

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Passenger or Driver: Can Gene Expression Profiling Tell Us Anything about LINE-1 in Cancer?

Stephen Ohms, Jane E. Dahlstrom and
Danny Rangasamy

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.73266>

Abstract

LINE-1 retrotransposons are expressed in epithelial cancers but not normal adult tissues. Previously, we demonstrated repression of cell proliferation, migration, and invasion genes in L1-reverse transcriptase-inhibited T47D cells, while genes involved in cell projection, formation of vacuolar membranes, and intercellular junctions were upregulated. Extending this, we examined microarray data from L1-silenced and Efavirenz-treated T47D cells by Weighted Gene Correlation Network Analysis and literature mining. Hub genes in the most significant module comparing L1-silenced and untreated controls included HSP90AB2p, DDX39A, PANK2, MT1M, and LIMK2. HSP90AB2p is related to HSP90, a master regulator of cancer, cancer evolvability and chemo-resistance. DDX39A is a known cancer driver gene while PANK2 and MT1M affect multiple pathways. LIMK2 and SYBL1 impact actin cytoskeletal dynamics and the cofilin pathway, cancer cell motility, and the epithelial-to-mesenchymal transition. Also affected were signal transduction, HIF1 pathways, iron/redox metabolism, stress granules and cancer stem cell-related metabolic reprogramming and the eIF4F translation initiation complex. Hub genes in other modules, including BTRC, MDM2, and FBXW7, stabilize oncoproteins like MYC, p53, and NOTCH1 or reflect CXCL12–CXCR4 signalling. Our findings support mounting evidence that L1 activity is a cause, rather than a consequence of oncogenesis, with L1 affecting the formation of cancer stem cells.

Keywords: LINE-1, breast cancer, cancer stem cells, CSC, WGCNA, module eigengene, stress granule, protein kinase R, proteomics, cancer driver genes, cancer evolvability, epithelial-mesenchymal transition, mesenchymal-epithelial transition, EMT, MET, LINE-1 ORF1 protein interactome, MYC Coding Region Instability Determinant (CRD), HSP90, ROS, iron

1. Introduction

Retrotransposons are mobile genetic elements that replicate through an RNA intermediate, which is copied into genomic DNA by a retrotransposon-encoded reverse transcriptase. Retrotransposons are classified into two subclasses, the long terminal repeat (LTR) elements (human endogenous retroviruses or HERVs) and non-LTR elements (long interspersed elements [LINEs], including LINE-1 (L1) elements, and short interspersed elements [SINEs], including SVA and Alu elements). L1 elements are the most prolific type of retrotransposon and can mediate insertional mutations and other forms of genome reorganization leading to several human disorders and genomic plasticity [1, 2]. There are approximately 7000 full-length L1 copies in the human genome, at least 100 of which are classified as highly active or retrotransposition-competent [3, 4]. An active L1 element is composed of a 5'-untranslated region containing an internal promoter, two open reading frames (ORF1 and ORF2), and a 3' poly-A tail. ORF1 encodes an RNA-binding protein with nucleic acid chaperone activity, while ORF2 encodes reverse transcriptase (RT) and endonuclease enzymes, required for reverse transcription and integration of the L1 RNA intermediate into new genomic sites [2].

It has long been speculated that somatic L1 insertions might drive tumorigenesis by activating oncogenes or inactivating tumor suppressor genes. This seems to be rare in practice, although the failure to detect frequent L1 retrotransposition in tumors may reflect the fact that sequencing traditionally focuses on exons, whereas L1 insertions may be capable of exerting effects when inserted into introns by creating new promoters, altering transcription, or creating new polyadenylation sites [5–7].

Although adult tissues do not normally express L1 ORF1 protein (ORF1p) [8, 9], many human neoplasms do express L1 RNA and proteins, including epithelial neoplasms [9–11], multiple myeloma, and leukemias [12, 13]. This topic has been the subject of numerous reviews, many of which are recent (listed in **Table 1**), indicating that the role of L1 in cancer is gaining ever-increasing attention.

In summary, while a clear correlation has been established between L1 and cancer, whether L1 expression and activity is a cause rather than a consequence of oncogenesis has been unclear. Probably, the strongest evidence that L1 drives cancer is the finding that L1 induces hTERT and ensures telomere maintenance in tumor cell lines [33]. L1 knockdown also leads to decreased cMyc and KLF4 mRNA and protein expression, two of the main transcription factors of telomerase, and changes in mRNA levels of other stem cell-associated proteins like CD44 and hMyb, with correspondingly reduced growth in spheroids. In addition, knockdown of KLF4 or cMyc decreases L1-ORF1 mRNA levels, suggesting specific reciprocal regulation with L1 [33].

Furthermore, L1 activity is dependent on phosphorylation of L1 ORF1p by the peptidyl prolyl isomerase 1 (Pin1) and is thus integrated with regulatory phosphorylation cascades [34]. This suggests that, like many pathogens, L1 can appropriate a major regulatory cascade of the host, and that competition for kinases by ORF1p could perturb signaling cascades.

Title	Reference
Transposable elements in cancer	[14]
The role of somatic L1 retrotransposition in human cancers	[15]
LINE-1 methylation level and prognosis in pancreas cancer: Pyrosequencing technology and literature review	[16]
Methylation levels of LINE-1 as a useful marker for venous invasion in both FFPE and frozen tumor tissues of gastric cancer	[17]
The function of LINE-1-encoded reverse transcriptase in tumorigenesis	[18]
The human long interspersed element-1 retrotransposon: An emerging biomarker of neoplasia	[19]
Links between human LINE-1 retrotransposons and hepatitis virus-related hepatocellular carcinoma	[20]
The connection between LINE-1 retrotransposition and human tumorigenesis	[21]
The reverse transcriptase encoded by LINE-1 retrotransposons in the genesis, progression, and therapy of cancer	[22]
Crossing the LINE toward genomic instability: LINE-1 retrotransposition in cancer	[23]
LINE-1 in cancer: Multifaceted functions and potential clinical implications	[24]
Regulatory roles of LINE-1-encoded reverse transcriptase in cancer onset and progression	[25]
LINE-1 hypomethylation in blood and tissue samples as an epigenetic marker for cancer risk: A systematic review and meta-analysis	[26]
L1 retrotransposons, cancer stem cells and oncogenesis	[27]
Clinical implications of the LINE-1 methylation levels in patients with gastrointestinal cancer	[28]
Long interspersed element-1 (LINE-1): Passenger or driver in human neoplasms?	[29]
The human L1 element: A potential biomarker in cancer prognosis, current status and future directions	[30]
L1 retrotransposon and retinoblastoma: Molecular linkages between epigenetics and cancer.	[31]
A role for endogenous reverse transcriptase in tumorigenesis and as a target in differentiating cancer therapy	[32]

The list above is the subset of the results returned by a search in PUBMED using the search term: ((LINE-1) AND cancer) AND review.

Table 1. Reviews of LINE-1 involvement in cancer.

Further evidence for an active role for L1 in cancer comes from studies with anti-retroviral drugs that target the reverse transcriptase of L1. Efavirenz is a first-line antiretroviral drug used in the treatment of HIV-1 but also reported to suppress the activity of L1-RT and, remarkably, to promote morphological differentiation in a range of cancer cell lines [35, 36]. In addition to these reports, in another study, we showed that RT expression is widespread in MCF7 and T47D breast cancer cells and decreased markedly after treatment with Efavirenz [11]. Both cell types showed significantly reduced proliferation, accompanied by cell-specific differences in morphology. MCF7 cells displayed elongated microtubule extensions that adhered tightly to their substrate, while T47D cells formed long filopodial projections. These morphological changes were reversible upon stopping RT inhibition, confirming their dependence on RT activity. Microarray gene expression profiling showed that genes involved in proliferation, cell migration, and invasive activity were repressed in RT-inhibited cells.

Concomitantly, genes involved in cell projection, formation of vacuolar membranes, and cell-to-cell junctions were upregulated.

Standard microarray or RNA-seq analyses seek to identify differentially expressed genes in which each gene is analyzed independently. This approach fails to use much of the information that is captured in the transcriptome profiling experiment, namely that the expression of many genes is correlated. Thus, WGCNA quantifies the correlations between individual pairs of gene expression profiles and also the extent to which any two genes are highly correlated with the same neighbors (called topological overlap). The underlying assumption is that the correlated gene profiles and genes that overlap topologically must reflect common regulatory mechanisms or biological function.

In gene networks, a gene that has many interactions with other genes is called a hub gene and usually plays an essential role in gene regulation and biological processes [37, 38]. Compared to standard gene-wise methods of analysis, WGCNA has the advantage of enabling the identification of these hub genes and, in addition, overcomes the problem of multiplicity of hypothesis testing. This is because the number of modules of co-expressed genes is far less than the number of genes on the microarray and a single consensus gene profile from each module is subjected to statistical testing in preference to individual genes. Another advantage of WGCNA is that hub genes and other interesting genes in a module that are relevant to the phenotype under investigation may not be differentially expressed and would escape notice in a conventional gene-wise analysis.

Motivated by our initial findings described above, we decided to reanalyze the transcriptome data in greater detail using the more powerful WGCNA method [39], combining the data from Efavirenz (Efa)-treated cells [11] with our unpublished microarray data from T47D cells subjected to L1-silencing mediated by siRNA. An additional reason for combining the data was that the reproducibility of the co-expressed gene modules found by WGCNA increases as the number of samples increases with 12–15 samples currently being regarded as the practical minimum.

2. Methods

The details of the gene expression profiling in Efa-treated T47D cells have been published previously [11]. The siRNA-treated T47D cells were treated and harvested at the same time to minimize batch effects. Briefly, total RNA was isolated from cells and labeled cDNA hybridized to Roche NimbleGen Human Whole Genome 12-plex arrays. Gene expression levels were calculated with NimbleScan Version 2.4. Relative signal intensities for each gene were generated using the Robust Multi-Array Average algorithm with quantile normalization and summarized by the median polish method with NimbleScan Version 2.4. The biological samples included four experimental groups (L1 silenced by siRNA (pUTR), controls with scrambled vector (pSM2), Efavirenz-treated (Efa), and dimethyl sulfoxide-treated controls (DMSO)). There were three replicate samples in each group. To calculate individual gene-wise p-values and fold changes for the contrasts between L1-silenced or Efavirenz-treated cells and untreated controls, the (Robust Multi-array Average) RMA-normalized

calls files were imported into Partek Genomics Suite v6.2 (St. Louis, Missouri, USA), and the log₂ gene expression values were analyzed with a one-factor ANOVA design: (Treatment—with four levels—"DMSO", "Efa", "pSM2", "pUTR"). Contrasts were calculated for pUTR versus pSM2 and Efa versus DMSO. 4951 probes passed a false discovery rate threshold of 0.001 for the pUTR versus pSM2 contrast and 9946 for the Efa versus DMSO contrast.

For the WGCNA analysis, the RMA-normalized calls files were imported into R (version 3.1.0) [40] as log₂ values, and a subset of the 10,000 most variable probesets was selected to remove noise genes (measured by variance of the expression values of each gene across the 12 samples). A weighted gene coexpression network was constructed using the WGCNA package. Plots of scale-free fit using the pickSoftThreshold and softConnectivity functions indicated that a reasonable scale-free fit could be achieved by setting the soft-thresholding power (beta, β) for network construction to 20. The other parameters used for the blockwiseModules function in WGCNA included a minimum module size of 40, and the dendrogram cut height for module detection set to 0.10 to define modules of co-expressed probesets. networkType was set to "signed," maxBlockSize was set to 10,000, and other parameters were left at their default values.

The statistical enrichment of the overlap between the genes in some modules and relevant gene lists identified in literature was calculated using an online program at http://nemates.org/MA/progs/overlap_stats.html which uses the hypergeometric distribution. This program calculates a representation factor, which is the number of overlapping genes between any two gene lists divided by the expected number of overlapping genes drawn from two randomly chosen gene lists of similar size and is a measure of the enrichment of a gene list with genes from another list. A representation factor > 1 indicates more overlap than expected between two independent groups. A genome size of 19,000 genes was used in all overlap calculations.

3. Results and discussion

3.1. Module discovery

Based on a correlation threshold, WGCNA assigns genes to modules (clusters) in which the expression of genes in a module varies in a similar manner across the different experimental conditions. The modules are labeled automatically by WGCNA with a color code according to the number of genes in the module: turquoise denotes the largest module, blue the next, then brown, green, yellow, etc. WGCNA identified 34 modules (excluding a gray module containing unassigned probesets) ranging in size from a darkmagenta module (58 probes) to a turquoise module (1359 probesets). For statistical analysis, each module is represented by a consensus profile of all the genes in the module, by default, the first principal component, to calculate a module eigengene. A one-factor ANOVA analysis was carried out on the module eigengenes in R using the same ANOVA design (Treatment—with four levels—"DMSO", "Efa", "pSM2", "pUTR") used for the gene-wise analysis in Partek. After correcting for multiplicity by multiplying all p-values by 34, the most significant module eigengene for the contrast between

L1-silenced and scrambled vector controls was for the darkmagenta module (**Figure 1**) with a Bonferroni-corrected p-value of $6.55E - 11$ (uncorrected p-value $1.93E - 12$) (**Table 2**). All genes in the darkmagenta module were downregulated in a range from -1.53 to -2.62 in the contrast between L1-silenced and scrambled vector controls (**Figure 2**). The most significant module eigengene for the contrast between Efavirenz-treated and DMSO controls was for the black module (Bonferroni-corrected p-value $1.15E - 09$) (**Figure 3**). Due to space limitations, the following results and discussion focus mainly on the darkmagenta module with references to genes in other modules (**Figures 4–8**) that can be linked in common pathways or processes to those in the darkmagenta module.

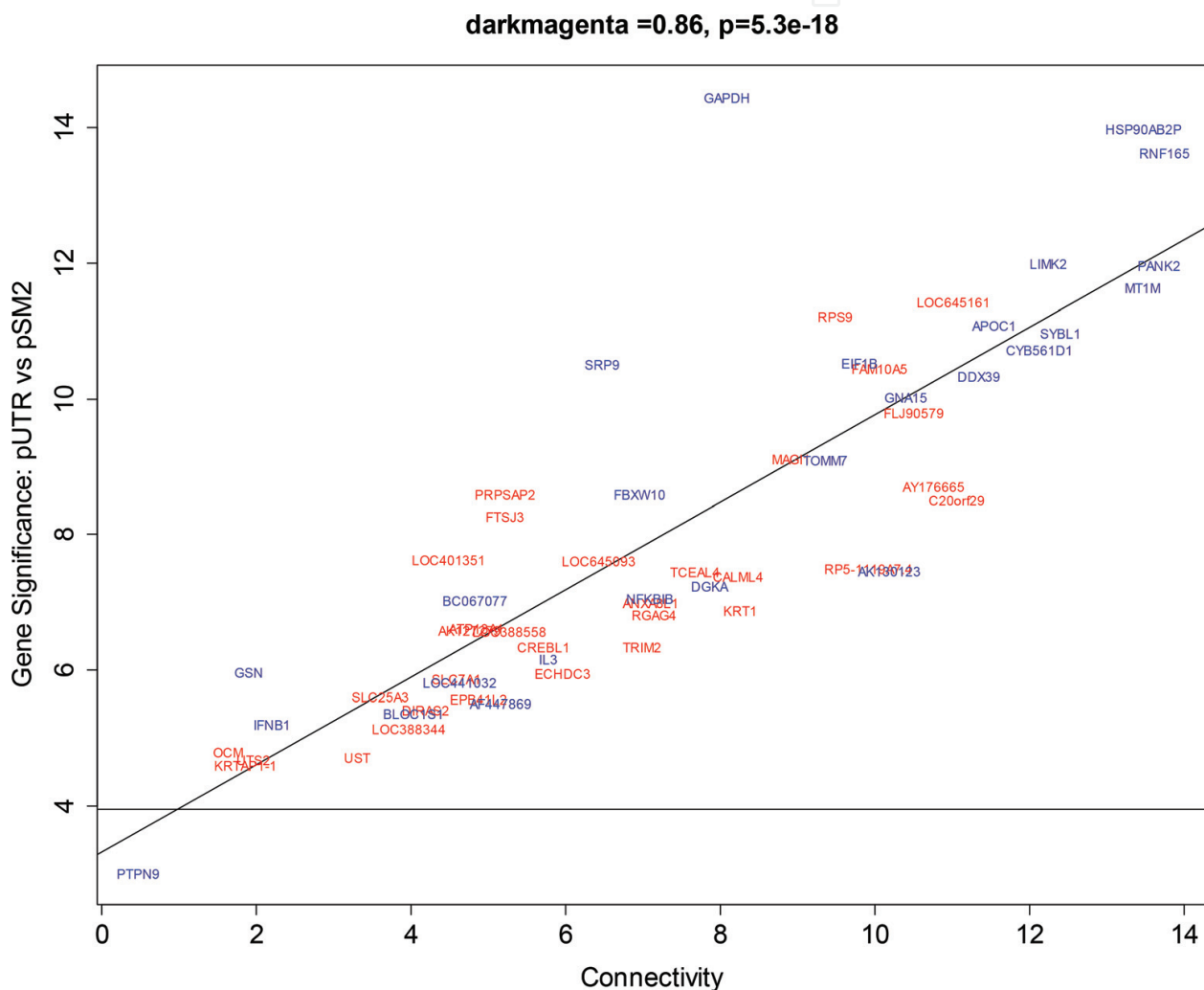


Figure 1. Scatterplot for the darkmagenta module. In Figures 1–8, genes specifically mentioned in the text are labeled blue, otherwise they are labeled red. The vertical axis (Gene Significance) is the $-\log_{10}(\text{p-value})$ for the contrast between pUTR versus pSM2 (**Figures 1, 2, 4–8**) or for Efavirenz versus DMSO (**Figure 3**). The intramodular connectivity for each gene is plotted on the horizontal axis. Genes with higher values of gene significance have smaller p-values in the gene-wise analysis in Partek Genomics Suite. Genes towards the right of the plots have higher intramodular connectivities and are hub genes. Intramodular connectivities were calculated with the WGCNA/intramodularConnectivity function from an adjacency matrix calculated by the WGCNA/adjacency function on the 10,000 most variable probes and with a soft thresholding power = 20. The horizontal line is the false discovery rate (FDR) 0.001 threshold calculated in Partek GS for the gene-wise ANOVA contrast. All genes above this line pass the FDR threshold at the 0.001 level. The plot was created with the WGCNA/verboseScatterplot function.

Module eigengene	pUTR vs. pSM2	Efa vs. DMSO	pUTR vs. pSM2 (Bonferroni)	Efa vs. DMSO (Bonferroni)
MEdarkmagenta	1.93E - 12	6.60E - 11	6.55E - 11	2.25E - 09
MEpurple	2.42E - 11	2.79E - 07	8.24E - 10	9.47E - 06
MEviolet	6.64E - 11	0.05308796	2.26E - 09	1.80E + 00
MEorange	8.03E - 11	0.01438891	2.73E - 09	4.89E - 01
MEwhite	1.38E - 10	1.16E - 09	4.68E - 09	3.95E - 08
MEroyalblue	1.85E - 10	5.02E - 11	6.31E - 09	1.71E - 09
MElightyellow	5.11E - 10	0.01025057	1.74E - 08	3.49E - 01
MEmagenta	1.09E - 09	1.21E - 08	3.69E - 08	4.10E - 07
MEdarkgreen	1.57E - 09	6.28E - 10	5.35E - 08	2.13E - 08
MEdarkgrey	2.73E - 09	5.28E - 08	9.29E - 08	1.80E - 06
MEsteelblue	7.19E - 09	1.05E - 07	2.45E - 07	3.57E - 06
MEdarkolivegreen	7.37E - 09	1.00E - 07	2.51E - 07	3.41E - 06
MEred	7.67E - 09	2.17E - 07	2.61E - 07	7.37E - 06
MElightgreen	7.98E - 09	0.000116041	2.71E - 07	3.95E - 03
MEcyan	1.02E - 08	0.001002342	3.47E - 07	3.41E - 02
MEgreenyellow	2.16E - 08	0.001305863	7.36E - 07	4.44E - 02
MEtan	4.37E - 08	1.36E - 08	1.49E - 06	4.64E - 07
MEyellow	6.60E - 08	2.77E - 10	2.24E - 06	9.40E - 09
MEgrey60	6.76E - 08	6.75E - 10	2.30E - 06	2.29E - 08
MEmidnightblue	9.07E - 08	0.1888604	3.08E - 06	6.42E + 00
MEdarkorange	6.92E - 07	9.11E - 09	2.35E - 05	3.10E - 07
MEblack	1.18E - 06	3.37E - 11	4.00E - 05	1.15E - 09
MElightcyan	2.67E - 06	2.93E - 10	9.08E - 05	9.95E - 09
MEgreen	5.77E - 06	5.31E - 10	1.96E - 04	1.80E - 08
MEsaddlebrown	1.15E - 05	1.44E - 09	3.89E - 04	4.88E - 08
MEblue	1.73E - 05	3.75E - 09	5.89E - 04	1.27E - 07
MEskyblue	4.73E - 05	9.32E - 10	1.61E - 03	3.17E - 08
MEturquoise	0.000172871	8.22E - 08	5.88E - 03	2.79E - 06
MEdarkred	0.00019623	4.79E - 11	6.67E - 03	1.63E - 09
MEpink	0.000201452	0.182879	6.85E - 03	6.22E + 00
MEsalmon	0.000286858	3.75E - 11	9.75E - 03	1.27E - 09
MEpaleturquoise	0.000292228	8.01E - 08	9.94E - 03	2.72E - 06
MEdarkturquoise	0.002355459	1.15E - 06	8.01E - 02	3.91E - 05
MEbrown	0.03581247	1.16E - 09	1.22E + 00	3.94E - 08

pUTR vs. pSM2 is the ANOVA contrast p-value for L1 silenced by siRNA versus scrambled vector controls. Efa vs. DMSO is the ANOVA contrast for Efavirenz-treated versus DMSO controls.

Table 2. Uncorrected and Bonferroni-corrected p-values for ANOVA contrasts for module eigengenes.

3.2. LINE-1 silencing affects HSP90, a master regulator of cancer

The most extreme outlier in the darkmagenta module is HSP90AB2P (**Figure 1**). Despite its classification as a pseudogene, the existence of this protein is supported by mass spectrometry evidence [41]. The parent gene, heat shock protein 90 (HSP90) is a ubiquitously expressed molecular chaperone representing 1–2% of all cellular protein that controls the folding, assembly, intracellular disposition, and proteolytic turnover of approximately 100 proteins, most of which are involved in signal transduction [42]. HSP90 proteins also stabilize and refold denatured proteins under stress, with two major cytosolic forms, an inducible form (HSP90AA1, a hub in the cyan module, **Figure 4**) and HSP90AB1, a constitutive form. Significantly, both HSP90AA1 and HSP90AB1 have been identified as members of L1 ORF2p complexes in isotopic differentiation of interactions as random or targeted (I-DIRT) affinity proteomics experiments and quantitative MS [43], thus supporting the presence of HSP90AB2P and

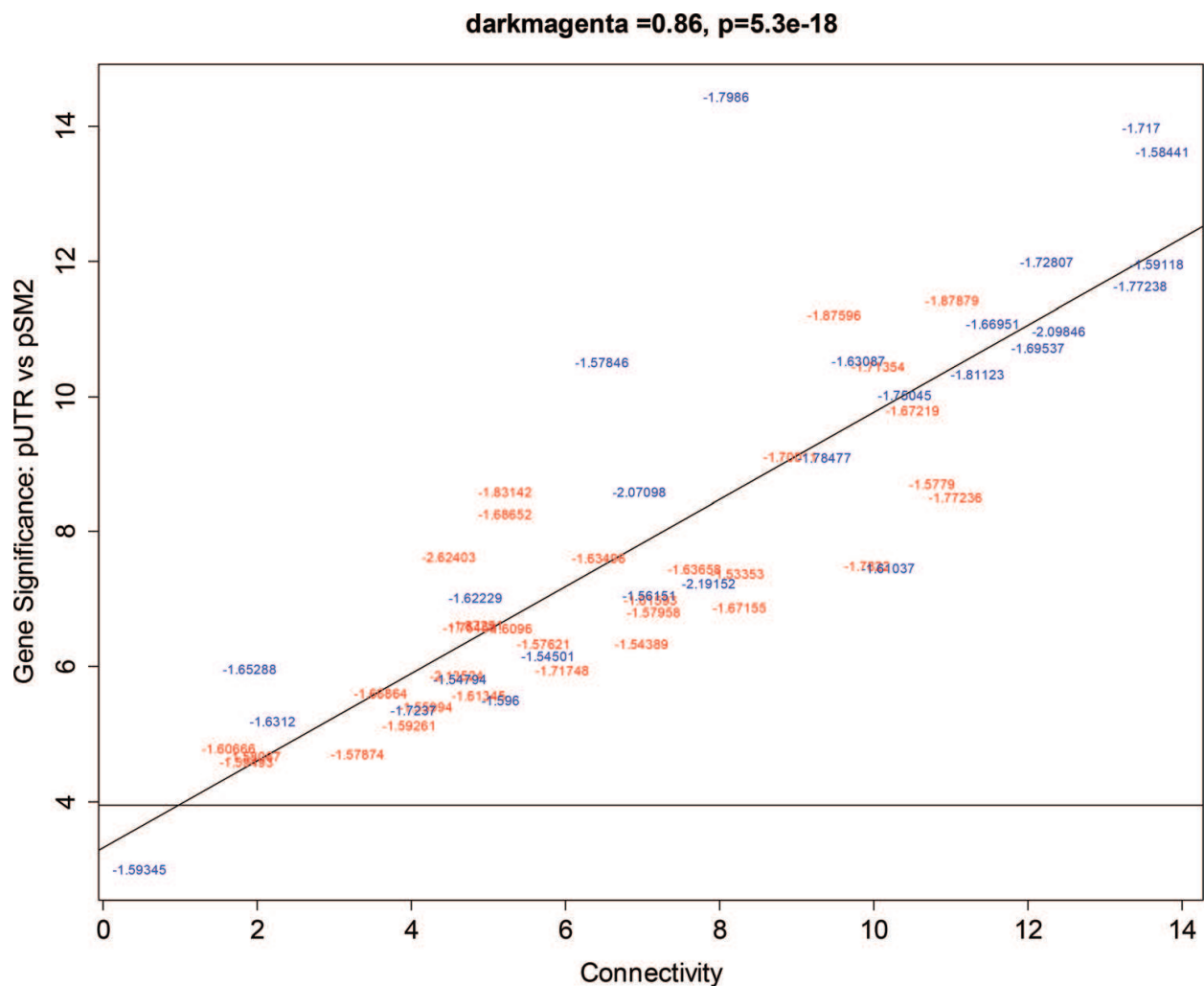


Figure 2. Similar plot to **Figure 1** but points are labelled with the fold-change for the gene in the comparison between pUTR and pSM2 (L1-silenced versus controls). All genes in the darkmagenta module are downregulated in a range from -1.53 to -2.62 for this comparison.



Figure 5. Scatterplot for the pink module. The pink module is enriched in genes from the LINE-1 ORF1 protein interactome (DDX21, NPM1, PABPC4, PTBP1, STAU1, STK38) with a representation factor of 3.2 and p-value <0.011.

including LINE-1, with the co-chaperone Fkbp6 having a critical role in delivering piRNAs to Miwi2 in the mouse [52].

3.3. LINE-1 silencing potentially affects the THOC/TREX nuclear export complex through DDX39A

DDX39A and SRP9 in the central region of the darkmagenta module plot (**Figure 1**) were among 96 proteins associated with the L1 ORF1p and its ribonucleoprotein identified by co-immunoprecipitation of tagged L1 constructs and mass spectrometry [53]. DDX39A (also known as DDX39 or URH49) is a member of the DEAD box RNA helicase family implicated in processes involving alteration of RNA secondary structure, including translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly. There are two closely related paralogs, DDX39A and DDX39B (also known as UAP56 or BAT1), both of

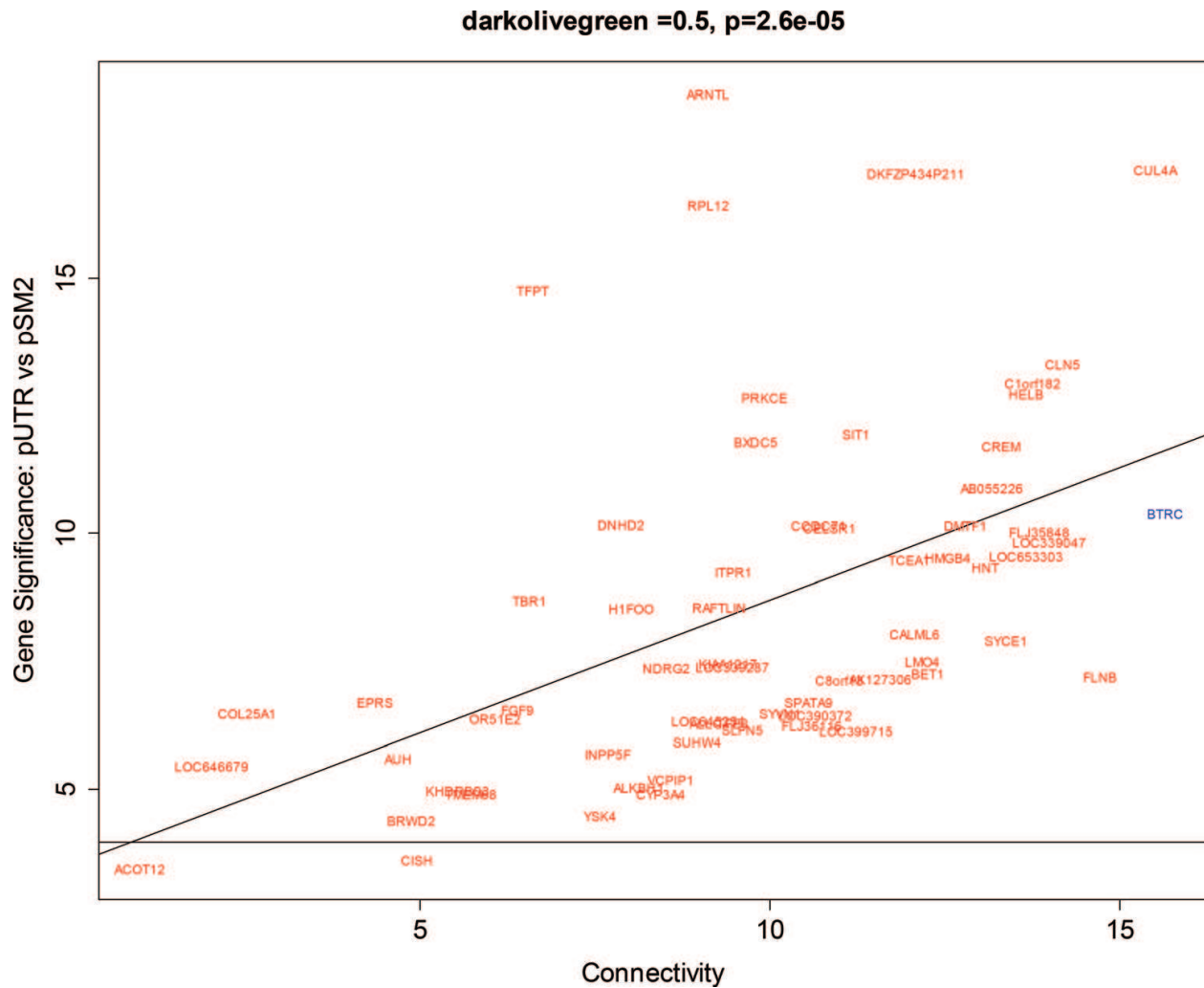


Figure 7. Scatterplot for the darkolivegreen module.

DDX39 has also been identified as a cancer driver gene in two studies. Firstly, DDX39 was identified as a marker predicting urinary bladder cancer progression by proteome analysis [60]. Secondly, DDX39 was identified as a key driver gene and anti-cancer drug target by data mining in the “Sanger Genomics of Drug Sensitivity in Cancer dataset from the Cancer Genome Project” [61]. This dataset contains gene expression levels, copy number, and mutation status for 654 cell lines and IC50 values of 138 anti-cancer drugs. The string-db network [62] of the potential driver genes with the highest 10 largest importance measures among the selected genes for each anti-cancer drug is shown in **Figure 9**.

Four of the markers identified by Kato et al. [60] (CCT4, IDH1, NPM1, YBX1) overlap the L1 ORF1p interactome resulting in a statistically significant overlap with a representation factor of 56.5 and $p < 5.893E-07$. There are also five overlaps between the L1 ORF1p interactome and the cancer driver gene set identified by Park et al. [61] (DDX39A, NPM1, PABPC4, TCP1, YBX1) resulting in a representation factor of 9.9 and $p < 1.528E-04$. This is, in itself, strong evidence for LINE-1 having an active rather than a passive role in cancer.

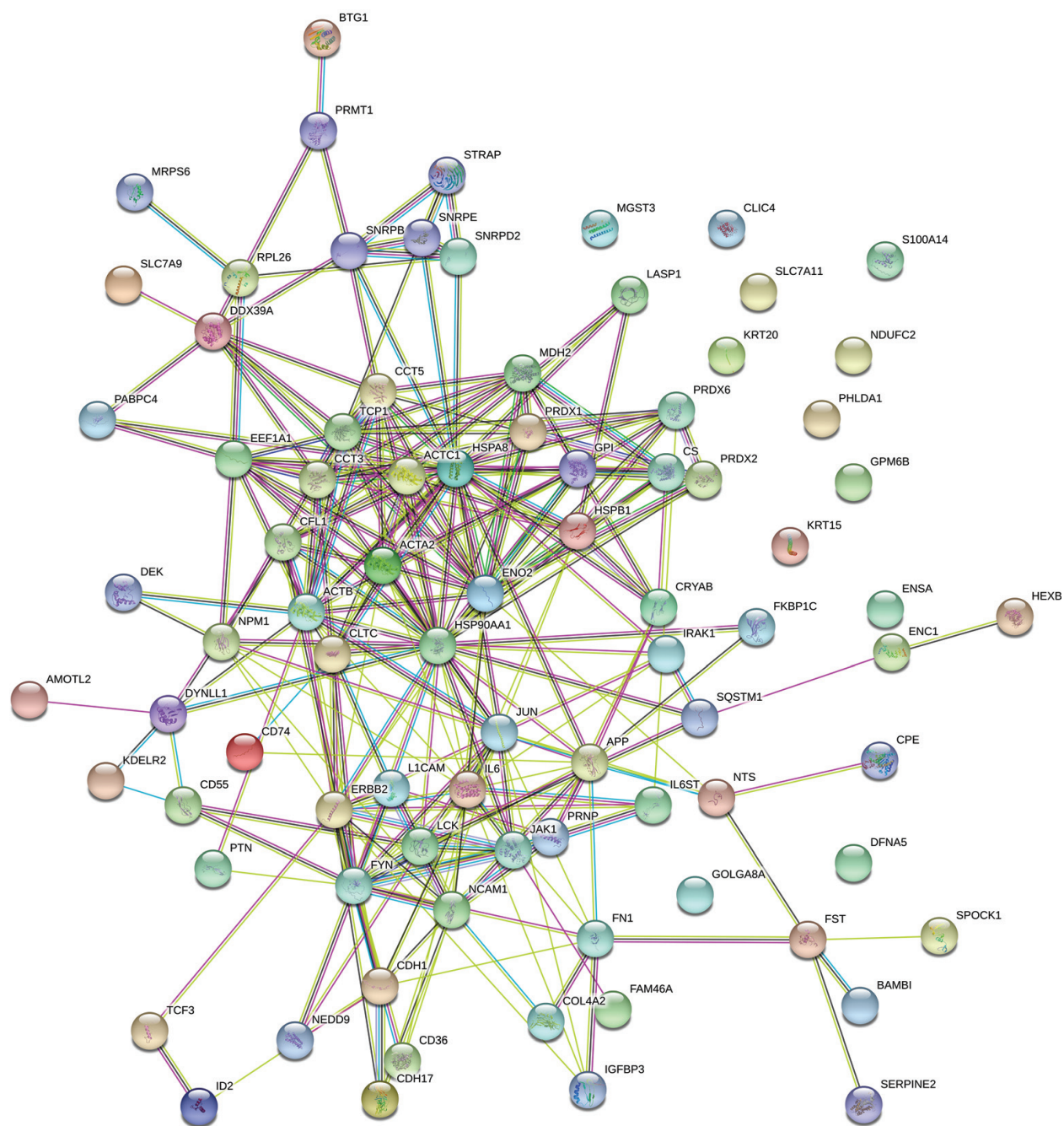


Figure 9. String-db gene network for cancer driver genes identified using data mining by Park et al. [61]. Network connections are based on known and predicted protein-protein interactions. Medium confidence interactions are shown. The network shows the central location of HSP90AA1 and ERBB2. Genes from the darkmagenta module and the LINE-1 ORF1p interactome are also present.

Mutations in the *Drosophila* PANK homolog (dPANK) lead to reduced CoA levels, impaired acetylation of histones leading to downstream epigenetic effects, and impaired acetylation and stability of tubulin [63].

PANK deficiency in *Drosophila* and human neuronal cell cultures leads to abnormalities in F-actin organization and abnormally high levels of phosphorylated cofilin (CFL1) (**Figure 9**),

a conserved actin filament severing protein. The increased levels of phosphorylated cofilin coincide with morphological changes in PANK-deficient *Drosophila* S2 cells and human neuronal SHSY-5Y cells with the latter also forming markedly fewer neurites in culture—a process that is strongly dependent on actin remodeling [63]. Cofilin also plays a critical role in breast cancer invasion and metastasis [64] with the cofilin pathway comprising a group of kinases and phosphatases that regulate cofilin and coordinately initiate actin polymerization and cell motility in response to stimuli in the microenvironment of mammary tumors.

Mutations in the human PANK2 gene lead to neurodegeneration with brain iron accumulation and are linked to changes in ferroportin expression, the only known protein to mediate the export of intracellular iron [65]. Downregulation of PANK2 by siRNA in HeLa cells leads to a 12-fold induction of ferroportin mRNA [66]. Ferroportin is strongly downregulated in breast cancer, possibly being required for phenotypic transitions occurring during metastasis [67]. High ferroportin gene expression identifies an extremely favorable cohort of breast cancer patients with a 10-year survival of >90% [68].

Iron-dependent oxidative demethylation mediated by the Jumonji family of enzymes is linked to the epigenetic regulation of cancer [69, 70]. H3K4 methylation is a key determinant of epithelial-mesenchymal plasticity, and loss of H3K4me3 correlates with poor survival in breast cancer [71]. In addition, the ten–eleven translocation (TET) enzymes promote the iron-dependent oxidative demethylation of 5-methylcytosine and regulate the epithelial-mesenchymal transition (EMT) and the reverse mesenchymal-epithelial transition (MET) [72–74]. Iron may also be directly involved in promoting selective oxidative demethylation of key DNA or histone residues in chromatin to control the epithelial-mesenchymal status in a dynamic manner.

Iron and iron-mediated processes appear to have a central role in the formation of breast cancer stem cells (CSCs) and to be potential therapeutic targets in breast CSCs [67]. Salinomycin and a derivative, Ironomycin, exhibit potent selective activity against breast CSCs *in vitro* and *in vivo*, by accumulating and sequestering iron in lysosomes [67]. Preferential iron trafficking also characterizes glioblastoma (GBM) stem-like cells [75]. GBM CSCs have been shown to potently extract iron from the microenvironment more effectively than other tumor cells and preferentially require the transferrin receptor and ferritin, two core iron regulators, to propagate and form tumors *in vivo*. Transferrin was the top upregulated gene compared with tissue-specific progenitors [75].

The presence of CYB561D1, a putative mitochondrial ferrireductase in the darkmagenta module close to PANK2 (**Figure 1**), further supports perturbation of iron-related metabolism by L1-silencing. A paralog, CYB561D2 (101F6), is highly expressed in lung tumor cell lines [76]. Its forced expression in NSCLC tumor cell lines or tumor xenografts significantly reduces cell viability by inducing apoptosis while lung metastases in nu/nu mice are also greatly reduced following systemic delivery of 101F6-encoding adenoviral vectors [77].

PANK2 also affects NADH levels [78, 79]. Hepatocytes from dKO PANK2 mouse pups cannot maintain NADH levels compared to wild-type hepatocytes [80]. In addition, induced pluripotent stem cell (iPSC)-derived neuronal models of PANK2-associated neurodegeneration reveal

mitochondrial dysfunction with activated NADH-related and inhibited FADH-related respiration, leading to increased reactive oxygen species generation and lipid peroxidation [78].

The link between CoA and NADH also supports an important role for PANK2 in the metabolism of breast CSCs. Reactive oxygen species (ROS) and ROS-dependent signaling pathways and transcriptional activities appear to be critical to both normal stem cell self-renewal and differentiation and to CSCs [81]. CSCs possess low levels of ROS but how they control ROS production and scavenging and how ROS-dependent signaling pathways contribute to CSC function remain poorly understood.

In close proximity to PANK2 in **Figure 1**, MT1M is a member of the metallothionein (MT) family; metallothioneins are small cysteine-rich proteins involved in metal metabolism and detoxification and redox metabolism. Metallothioneins may form a critical surveillance system protecting cells from damage caused by electrophilic carcinogens [82]. However, several studies suggest that metallothioneins have wider roles, contributing to numerous fundamental carcinogenic processes, including proliferation, survival, metabolism, invasion, and metastasis [83, 84].

Metallothionein expression is also strongly associated with tumor grade in breast, ovarian, uterine, and prostate cancers [85].

Hypoxia-inducible factor-1 (HIF-1 α) can co-activate MT gene transcription by interacting with the metal-responsive transcription factor (MTF1) in hypoxic conditions increasing the biological aggressiveness of cancer cells [86, 87]. Conversely, metallothioneins can increase HIF-1 α transcriptional activity by suppressing ROS accumulation or activating the ERK/mTOR pathway [88, 89]. Also, even though MTF1 is not inducible by iron, expression of ferroportin is induced directly via MTF1 [90]. HIF-1 α also transcriptionally activates SLUG expression in hypoxic conditions [91, 92], and because upregulation of HIF-1 α and metallothionein expression is self-reinforcing, MT1M may also affect SLUG expression. SLUG is a member of the SNAIL superfamily of zinc finger transcriptional factors involved in the EMT. SLUG expression correlates with reduced cell adhesion, increased cell migration and invasion, and biological aggressiveness in several tumor types including breast cancer [93, 94].

While not a hub gene, GAPDH is the most significantly differentially expressed gene in the darkmagenta module (**Figure 1**). Overexpression of GAPDH occurs in diverse human cancers. Several cancer-related factors, such as insulin, HIF-1, p53, nitric oxide (NO), and acetylated histones, modulate GAPDH gene expression and affect GAPDH protein function [95]. In addition to its role in glycolysis, in which it catalyzes the oxidation and phosphorylation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate in conjunction with NAD⁺, GAPDH is a key mediator of oxidative stress responses, involving GAPDH nuclear translocation and induction of cell death [96]. GAPDH also inhibits telomerase activity and induces breast cancer cell senescence [96].

3.5. LINE1-silencing affects genes involved in MET-related metabolic reprogramming

The reprogramming of somatic cells to iPSCs by transgene expression of the transcription factors Oct4, Sox2, Klf4, and Myc triggers a mesenchymal-epithelial transition (MET) [97]. This

transformation is promoted by the TET enzymes and blocked by kinase-dependent cytoskeletal reorganization [98]. Two closely associated hub genes in the darkmagenta module (**Figure 1**), LIM Domain Kinase 2 (LIMK2) and Apolipoprotein C1 (APOC1), have roles in the MET, with the presence of APOC1 also suggesting the involvement of TET1. The TET proteins are DNA hydroxylases that mediate oxidation of methylcytosines and thus regulate hypoxia-sensitive gene expression. Among its many actions, TET1 regulates the hypoxia-induced EMT by acting as a co-activator of genes involved in cholesterol metabolism including APOC1 [73]. Significant changes in APOC1 expression are seen in leukemia cell lines in the NCI60 cancer cell line collection [99, 100], while APOC1 is highly expressed at the protein level and protects pancreatic cancer cells from apoptosis [101]. In addition, APOC1 is highly expressed in late-stage lung cancer [102] and is also one of a small number of genes undergoing late-stage upregulation downstream of KLF4 during the metabolic shift that facilitates reprogramming during the generation of iPSCs in an SeVdp(KOSM)-based system [103].

3.6. LINE-1 silencing targets DICER by acting through miR-103/107 embedded in the PANK2 gene

In addition to their central role in metabolism, the PANK1–3 genes contain the microRNAs, miR-103 and miR-107, in their intronic regions, with PANK1, 2, and 3 corresponding to pri-miR-107, pri-miR-103-2, and pri-miR-103-1, respectively. Expression of miR-103/107 has been shown to parallel that of the PANK genes in a series of cell lines and in normal human tissues [104]. Furthermore, miR-103/107 are predicted bioinformatically to affect multiple mRNA targets in pathways that involve cellular acetyl-CoA and lipid levels and thus to act synergistically with their host genes [105].

Although specific microRNAs can be upregulated in cancer, global miRNA downregulation is a common trait of human malignancies. This can be attributed, at least in part, to miR-103/107, which have been shown to target the 3'-UTR of Dicer leading to its downregulation and, in turn, to global downregulation of microRNA expression [106]. In human breast cancer, high levels of miR-103/107 are associated with metastasis and poor outcomes and this has been attributed to the miR-103/107-Dicer axis controlling epithelial plasticity and induction of the EMT, in part via regulation of miR-200 [106].

3.7. LINE-1 silencing is linked to the mitophagy-driven regulation of stem cell fate through TOMM7

The presence of Translocase Of Outer Mitochondrial Membrane 7 (TOMM7) in the darkmagenta module (**Figure 1**) is further evidence of L1 having an impact on cancer cell metabolism acting through HIF1 α . TOMM7 encodes a member of the TOM pre-protein translocase complex of the outer mitochondrial membrane, the main entry portal for protein precursors from the cytosol into mitochondria.

TOMM7 has a crucial role in mitophagy, the autophagic elimination of damaged mitochondria that has a role regulating stem cell fate [107]. Mitophagy is regulated by the PTEN-induced putative kinase 1 (PINK1). TOMM7 stabilizes PINK1 on the outer mitochondrial

membrane, and accumulation of PINK1 bound to the TOM complex is completely blocked by the loss of TOMM7 from the TOM complex [108]. PINK1 loss-of-function compromises both mitochondrial autophagy and oxidative phosphorylation and reprograms glucose metabolism through HIF1 [109]. Pink1 deficiency also stabilizes HIF1 α in cultured mouse embryonic fibroblasts and primary cortical neurons as well as in vivo [109]. This effect, mediated by mitochondrial ROS, leads to upregulation of the HIF1 target, PDK1 (pyruvate dehydrogenase kinase-1), which inhibits pyruvate dehydrogenase (PDH) activity. HIF1 α stimulates glycolysis in the absence of Pink1, and the promotion of glucose metabolism by HIF1 α stabilization is required for cell proliferation in Pink1^{-/-} mice. Thus, it is possible that loss of Pink1 reprograms glucose metabolism through HIF1 α , sustaining increased cell proliferation.

Independent support for the presence of TOMM7 in the darkmagenta module comes from an I-DIRT affinity proteomics study of L1 interactors [110]. TOMM40, another member of the TOM complex, was one of 37 high-confidence L1 ORF2-interactors in addition to Translocase Of Inner Mitochondrial Membrane 13 (TIMM13), a member of the TIMM family of proteins, that import proteins from the cytoplasm into the mitochondrial inner membrane in conjunction with the TOM complex.

3.8. LINE-1 silencing affects cytoskeletal dynamics and the MET through LIMK2, GSN, SYBL1, BLOC1S1, and RNF165

LIMK2 (darkmagenta module, **Figure 1**) has a key role in the MET, controlling the depolymerization of filamentous actin, by phosphorylating the actin stabilizer, cofilin. LIMK2 is one of the two kinases that have been shown to phosphorylate cofilin and stabilize actin stress fibers in fibroblasts, thus blocking the MET and preventing iPSC generation from mouse embryonic fibroblasts or human fibroblasts [98]. In the MET, the actin cytoskeleton is reorganized from actin stress fibres to cortical actin, the expression of mesenchymal transcription factors such as Zeb1 and Snai1 is lost, and the cells establish tight and adherens junctions stabilized by Par3/ZO-1 or E-cadherin [111].

Gelsolin (GSN) (lower left in darkmagenta module, **Figure 1**) is another key regulator of actin filament assembly and disassembly. Gelsolin is highly expressed at tumor borders infiltrating into adjacent liver tissues, contributes to lamellipodia formation in migrating cells, and induces tumor invasion by modulating the urokinase-type plasminogen activator cascade [112].

LIMK2 also acts with SYBL1 (darkmagenta module) in the assembly and maturation of invadopodia. Invadopodia are actin-rich protrusions that degrade extracellular matrix and are required for penetration through the basement membrane, stromal invasion, and intravasation. SYBL1 encodes VAMP-7, a transmembrane protein from the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family. VAMP-7 localizes to late endosomes and lysosomes and is involved in the fusion of transport vesicles to their target membranes. MT1-MMP is delivered by the IQGAP1-WASH-exocyst complex and fuses to the membrane via VAMP-7, resulting in matrix degradation [113].

Biogenesis of Lysosomal Organelles Complex 1 Subunit 1 (BLOC1S1) (darkmagenta module, **Figure 1**) is a component of the ubiquitous BLOC1 multisubunit protein complex required for the biogenesis of specialized organelles of the endosomal-lysosomal system, including melanosomes and platelet dense granules. Loss of BLOC1 function results in downregulation of the actin-related protein-2/3 complex (Arp2/3), a seven-subunit protein complex that plays a major role in the regulation of the actin cytoskeleton. This complex is present in cellular regions characterized by dynamic actin filament activity, including the leading edges of motile cells in lamellipodia, and also has a role in invadopodia [114]. The Arp2/3 complex is also potently activated by WASH [115].

The presence of RNF165/ARKL2 as a hub gene in the darkmagenta module (**Figure 1**), in the context of changes in expression of actin-related genes, is consistent with a bone morphogenetic protein (BMP)-driven MET. BMP has a key role in the induction of the MET [116] and RNF165/ARKL2 is an E3 ubiquitin-protein ligase that regulates motor axon elongation downstream of BMP [117]. A close homolog, RNF111/Arkadia is a key component of TGF β signaling [118] and amplifies TGF β and BMP signaling through degradation of the inhibitory Smad7. Aberrant RNF111/Arkadia activity occurs in clear-cell renal-cell carcinoma, colorectal cancer, and non-small cell lung cancer [119–122]. In contrast, not a great deal is known about RNF165 outside the nervous system, although it appears to have a significant role in metastatic prostate carcinoma [123].

3.9. LINE-1 affects stress granule formation through SRP9

The signal recognition particle (SRP) is a cytoplasmic ribonucleoprotein consisting of six polypeptides and a 300-nucleotide (7SL) RNA molecule. SRP9, a key member of the SRP is a member of darkmagenta module (**Figure 1**), while another member, SRP14, is a member of the L1 ORF1p interactome. The SRP9 and SRP14 polypeptides form a heterodimer and bind to the 3' and 5' ends of the SRP 7SL RNA. The SRP functions in the co-translational targeting of secretory and membrane proteins to the rough endoplasmic reticulum by complexing with ribosomes associated with the membrane of the RER via its receptor, SRPR, a hub gene in the pink module (**Figure 5**).

Remarkably, the Alu family of SINES is thought to have originated from a 7SL RNA gene early in primate evolution [124] and subsequently amplified by retrotransposition so that over 1 million copies are now present in the human genome [125]. Binding of the SRP 9/14 proteins to the RNA of Alu elements precedes and is likely to be necessary for efficient L1-mediated Alu retrotransposition [126, 127].

In addition, the SRP9/14 heterodimer can bind to cytoplasmic Alu RNA and 40S ribosomal subunits in a pathway involving the formation of stress granules (SGs) [128]. Cellular stress triggers the formation of dense cytosolic aggregations that sequester mRNA, 40S ribosomal subunits, initiation factors, and RNA-binding and signaling proteins to promote cell survival. SRP9/14 localizes to SGs following arsenite or hippuristanol treatment. The localization and function of SRP9/14 in SGs is mediated by direct binding to 40S ribosomal subunits. Binding of SRP9/14 to 40S or Alu RNA is mutually exclusive indicating that the heterodimer alone is

bound to 40S in SGs and that Alu RNA may competitively regulate 40S binding. Following resolution of stress, cells actively increase cytoplasmic Alu RNA levels to promote disassembly of SGs by disengaging SRP9/14 from 40S [128].

The involvement of stress granules in tumor initiation in breast cancer cells was discovered by screening for intracellular proteins enhancing the effect of chemotherapeutic agents on TIC-enriched breast cancer cells [129]. This screen identified 44 proteins that interacted with the lead compound, C108, including the stress granule-associated protein and GTPase-activating protein (SH3 domain)-binding protein 2 (G3BP2). G3BP2 was shown to regulate breast tumor initiation through the stabilization of squamous cell carcinoma antigen recognized by T cells 3 (SART3) mRNA, leading to increased expression of the pluripotency transcription factors Oct4 and Nanog. THOC6, an interaction partner of DDX39B in the THO complex and involved in the nuclear export of pluripotency-related transcripts, was also among the 44 interacting partners of C108.

At least two genes in the darkmagenta module (**Figure 1**) are linked to the C108 protein interactome, thus supporting the involvement of this module in SG formation. PTPN9 is present in C108 protein interactome, while AK130123 is highly similar to PPP2R2A, whose gene product interacts with those of PPP2R1A and PPP2R1B (present in the C108 protein interactome).

Another three interaction partners of C108 (IGF2BP1, IGF2BP2, and PABPC1) and SART1, but not SART3, are also present in the L1 ORF1p interactome. The degree of overlap between these two interactomes is statistically significant with a representation factor of 12.6 and $p < 0.002$. L1 ORF1 protein has, in fact, been shown by yeast two-hybrid screening to localize in stress granules with other RNA-binding proteins, including components of the RISC complex [130].

3.10. LINE-1 is likely to promote the cancer stem cell phenotype through SART1 and SART3

Although not members of the darkmagenta or any other module, SART1/TIP110, a member of the L1 ORF1p interactome and the functionally related SART3 implicate the L1 ORF1 protein in promotion of the cancer stem cell phenotype. SART1 (also known as U4/U6.U5 Tri-SnRNP-Associated Protein 1) encodes two proteins, the SART1(800) protein expressed in the nucleus of the majority of proliferating cells and the SART1(259) protein expressed in the cytosol of epithelial cancers. The SART1(259) protein is translated by -1 frameshifting during post-transcriptional regulation. SART1(259) plays an essential role in mRNA splicing by recruiting the tri-snRNP to the pre-spliceosome during spliceosome assembly. In contrast, SART3 associates transiently with U6 and U4/U6 snRNPs during the recycling phase of the spliceosome cycle. As mentioned before, stabilization of SART3 mRNA leads to increased expression of the pluripotency transcription factors, Oct-4 and Nanog [129]. SART3 also regulates OCT4 splicing in hESCs [131].

A recent proteomics study identified 13 SART3/TIP110-interacting cellular proteins, 5 of which are also present in the L1 ORF1p interactome [132]. This degree of overlap is highly significant with a representation factor of 76.1 and a p -value $< 3.694E-09$. These observations suggest that L1 affects SART3 in some way, thus implicating L1 in SART3-mediated breast cancer initiation.

Like SART3, SART1(800) also has fundamental roles in the formation of cancer stem cells. SART1(800), also known as hypoxia-associated factor (HAF), is overexpressed in a variety of tumor types. HAF is an E3 ubiquitin ligase that binds to and ubiquitinates HIF-1 α by an oxygen- and pVHL-independent mechanism, targeting HIF-1 α for proteasomal degradation [133]. HAF expression lowers HIF-1 α levels and decreases HIF-1 transactivating activity. HAF also binds to HIF-2 α but does not lead to its degradation and instead *increases* HIF-2 transactivating activity. Thus, HAF expression switches the hypoxia response of the cancer cell from HIF-1 α - to HIF-2 α -dependent transcription of genes such as MMP9 and OCT-3/4. This switch by HAF promotes the cancer stem cell phenotype and invasion, resulting in highly aggressive tumors in vivo [134].

3.11. LINE-1 silencing affects cancer-related signal transduction pathways by downregulating DGKA and GNA15

The presence of diacylglycerol kinase alpha (DGKA) and G protein subunit alpha 15 (GNA15) in the darkmagenta module (**Figure 1**) implicates LINE-1 silencing in affecting signal transduction pathways. Increasing evidence points to DGKA (DGK α) being a major node in oncogenic signaling [135]. DGKA converts diacylglycerol (DAG) to phosphatidic acid (PA), with both being critical lipid second messengers found in the plasma membrane. DGKA activity terminates DAG signaling and has been linked to activation of NF- κ B, HIF-1 α , c-Met, ALK, and VEGF [136]. DAG, in turn, binds directly to protein kinase C and D family members, to Ras family members, and to diacylglycerol kinase family members, while PA controls the activity of mTOR, Akt, and Erk.

DGKA plays an important role in the spread and invasion of breast cancer cells [137]. Among the microenvironment signals sustaining cancer cell invasiveness, stromal cell-derived factor-1 α (SDF-1 α , or CXCL12) plays a major role in several cancers, including breast cancer [138]. SDF-1 α is a chemokine secreted by tumor-associated fibroblasts and bone marrow stromal cells, which by activating its CXCR4 receptor (tan module, **Figure 6**), promotes migration and invasion of malignant cells and their homing to target organs [139, 140]. Following SDF-1 α stimulation, DGKA is activated and localized at cell protrusions, promoting their elongation and mediating SDF-1 α -induced MMP-9 metalloproteinase secretion and matrix invasion. PA generated by DGKA promotes recruitment of atypical PKCs (protein kinase C's) to cell protrusions or ruffling sites, which play an essential role by promoting Rac-mediated protrusion elongation and localized recruitment of β 1 integrin and MMP-9. Moreover, DGKA activity sustains the pro-invasive activity of metastatic p53 mutations, by promoting the recycling of α 5 β 1 integrin to invasive protrusions in tridimensional matrix [141].

GNA15 (also known as G15, G α 15 or GNA16) is a heterotrimeric G protein selectively expressed in immature hematopoietic and epithelial cells with high renewal potential. GNA15 is notable for its ability to bypass the usual selectivity of receptor G-protein interactions and to non-selectively couple structurally and functionally diverse receptors to phospholipase C [142]. Following activation of GPCRs, rapid desensitization of receptor responsiveness normally prevents uncontrolled signaling and is initiated by phosphorylation of the receptor by GPCR kinases [143, 144] followed by uncoupling of GPCR-G protein interactions mediated

by β -arrestin protein family members [145, 146]. Intriguingly however, GNA15 is not affected by GPCR desensitization. In certain cell lineages, GNA15 amplifies incoming stimuli regardless of β -arrestin-induced desensitization, thus promoting sustained activation of its downstream effectors, including key players in cancer signal transduction such as PKD1, Ras, Raf, PI3K, MEK, PKCs, and STATs [147–150]. Based on its resistance to desensitization and extraordinarily poor coupling selectivity [147], GNA15 may promote unconventional stimulation based on prolonged auto/paracrine activation of GPCRs. These may include GPCRs known for supporting the immature stages of pancreatic cancer, such as CXCR4 [151, 152], S1PRs [153–155], Frizzled [156, 157], and Smoothed (SMOH) [158–160].

GNA15 was recently identified in a three gene signature highly expressed in a leukemic stem cell-enriched CD34 + cell fraction in normal karyotype acute myeloid leukemia [161]. Ectopic expression of GNA15 is also found in pancreatic carcinoma [160]. In contrast, GNA15 mRNA and protein expression were found to be severely downregulated in a panel of non-small cell lung cancer cell lines and in human lung adenocarcinoma and squamous carcinoma patients [162]. Additionally, GNA15 has been identified as a regulator of non-small cell lung cancer cell proliferation and anchorage-independent cell growth [162].

3.12. Genes involved in protein kinase R stress signaling are enriched in the darkmagenta module

We uploaded the gene list from the darkmagenta module to the MetaCore web server (Clarivate Analytics; <https://clarivate.com/>) to search for enriched cellular pathways. The most significant pathway identified was that of “Apoptosis and survival_Role of PKR in stress-induced apoptosis” with a raw p-value = 4.925E-6 and a FDR-corrected p-value = 8.126E-4. The darkmagenta module contains 3 of the 53 genes in this pathway. These are NFKB1B, IFNB1, and AK130123 (a probable transcript variant of PPP2R2A). Although not identified as a member of this pathway, IL3 (also present in the darkmagenta module) appears to positively regulate protein synthesis by inducing the inactivation of PKR via a growth factor signaling pathway.

Protein kinase R (PKR) (also known as eukaryotic translation initiation factor 2 alpha kinase 2/EIF2AK2) is a serine/threonine protein kinase that is activated by autophosphorylation after binding to dsRNA. By this mechanism, PKR inhibits the replication of a wide range of DNA and RNA viruses by phosphorylating the alpha subunit of eukaryotic initiation factor 2 (EIF2S1/eIF2 α), a central node of the cellular response to stress signals. This impairs the recycling of EIF2S1 between successive rounds of initiation leading to inhibition of translation, which eventually results in shutdown of cellular and viral protein synthesis.

Stress-induced phosphorylation of EIF2S1 also induces stress granule assembly by preventing or delaying translational initiation and, additionally, is involved in the restriction of LINE-1 retrotransposition by SAMHD1. The HIV-1 restriction factor SAMHD1 can negatively modulate retrotransposition of LINE-1 by a mechanism that involves sequestration of L1 RNP in stress granules [163]. SAMHD1 promotes the formation of these stress granules by inducing phosphorylation of EIF2S1 and disrupting the interaction between eIF4A and eIF4G [163].

In addition to its role in stress granule formation, PKR phosphorylates p53/TP53, PPP2R5A, DHX9, ILF3, and IRS1 with DHX9 and ILF3 being members of the LINE-1 ORF1p interactome. Either as an adapter protein and/or via its kinase activity, PKR can also regulate the p38 MAP kinase, NFkB, and insulin signaling pathways and transcription factors (JUN, STAT1, STAT3, IRF1, ATF3) involved in the expression of genes encoding pro-inflammatory cytokines and interferons. PKR also has a role in the regulation of the cytoskeleton by binding to Gelsolin (GSN; darkmagenta module, **Figure 1**), sequestering the protein in an inactive conformation away from actin [164].

The downregulation of NFKBIB in the darkmagenta module suggests activation of NFkB signaling. Hyperactivation of NFkB induces the expression of stemness-associated genes and inflammatory genes in CSCs but this is likely to be context-dependent involving Toll-like receptor signaling and saturated fatty acids [165, 166].

3.13. LINE-1 silencing affects the initiation, elongation, and termination steps of protein translation

Dysregulation of three of the four major steps of mRNA translation: initiation, elongation, and termination, has been implicated in the development and progression of cancer. In addition to the role of PKR signaling in initiation mentioned above, several genes in the darkmagenta module can be directly linked to these steps as can several members of the L1 ORF1p interactome.

Elevated protein synthesis arises as a consequence of increased signaling flux channeled to eIF4F, the key regulator of the mRNA-ribosome recruitment phase of translation initiation and a critical nexus for cancer development. The eIF4F complex is a trimeric complex consisting of the eIF4E cap-binding protein, the eIF4G scaffold protein, and the eIF4A helicase and is subject to regulation by major oncogenic pathways, including the PI3K/AKT/mTOR and MAPK cascades [167]. At least three members of the L1 ORF1p interactome (eIF4B, PABPC1, and PABPC4) interact with eIF4A [167]. In addition, based on a string-db [62] analysis by us of the LINE-1 ORF1p interactome members including eIF4E, there is suggestive literature evidence for interactions between other components of the L1 ORF1p (PCBP2, LARP1, SSB, DDX39A, RNMT, HNRNPA1, and PCBP2) and eIF4E (data not shown). In addition, eIF1B, a highly connected gene in the darkmagenta module (**Figure 1**), is a key player in start codon selection, a critical step in translation initiation that sets the reading frame for decoding [168].

EEF1A1P9 or EEF1AL7 (LOC441032 in the darkmagenta module, **Figure 1**) is a pseudogene related to eukaryotic translation elongation factor 1A1 (eEF1A1/EEF1- α 1), an isoform of eEF1A. eEF1A is a protein subunit of the eukaryotic translation elongation 1 (eEF1) complex, which is composed of eEF1A, valyl-tRNA, and the eEF1B complex, comprising eEF1G, eEF1B, and eEF1D. Overexpression of EEF1D/eEF1 δ in cadmium-transformed Balb/c-3T3 cells in conjunction with eIF3 is a major mechanism responsible for cell transformation and tumorigenesis induced by cadmium [169]. In addition to eEF1A's canonical role in translational elongation, eEF1A has a growing list of functions beyond protein synthesis, including protein degradation [170, 171], apoptosis [172, 173], nucleocytoplasmic trafficking [174], heat shock [175], and

multiple aspects of cytoskeletal regulation [176]. eEF1A1 may also mediate turnover of the LINE-1 restriction factor, SAMHD1, by targeting it to the proteasome for degradation [177].

While translation termination is generally not considered a major target of tumorigenesis, eukaryotic release factors such as AF447869/GSPT1/eRF3 (darkmagenta module, **Figure 1**) are implicated in gastric cancer [178]. GSPT1/eRF3 is also involved in the regulation of cytoplasmic mRNA decay in association with Poly(A)-binding protein (PABP), two isoforms of which, PABPC1 and PABPC4, are present in the L1 ORF1p interactome. GSPT1 also has a role in nonsense-mediated decay [179].

There are five known GSPT1/eRF3a human alleles, one of which has been correlated with increased cancer risk in several studies and which may act by decreasing the binding affinity of GSPT1 for PABP [180]. Alternatively, GSPT1/eRF3 may be involved in tumorigenesis as a result of its non-translational roles, which affect cell cycle dysregulation, apoptosis, and transcription [178].

3.14. E3 ubiquitin protein ligases that affect oncoprotein stability are hub genes in several modules

Proteins that promote cell proliferation must be expressed in a controlled manner but also efficiently degraded. A major pathway for such targeted protein degradation is the ubiquitin-proteasome system (UPS), and oncoproteins that drive tumor development are often deregulated and stabilized in malignant cells. Several E3 ubiquitin protein ligases targeting oncoproteins are hub genes in other modules, including BTRC (a hub gene in the darkolivegreen module, **Figure 7**, fold change -1.68x downregulated in L1-silenced versus controls) and FBXW11 and FBXW7 (hub genes in the pink module (**Figure 5**), although neither are differentially expressed in L1-silenced versus controls). FBXW10 and BC067077 /MDM2, although not hub genes, are present in the darkmagenta module (**Figure 1**).

A number of proteins driving the development and progression of cancer are direct or indirect targets of the UPS. For example, FBXW7 (FBW7 or F-box and WD repeat domain containing 7 E3 Ub protein ligase) promotes ubiquitination and proteasomal degradation of mTOR [181]. This leads to breast cancer suppression in cooperation with PTEN. BTRC also regulates mTOR activity through the targeted degradation of DEP domain-containing mTOR-interacting protein (DEPTOR), an inhibitor of both mTORC1 and mTORC2 [182]. NOTCH signaling is involved in the short-range communication between neighboring cells, and its activation plays a key role in cancer progression. NOTCH receptors are regulated by multiple E3s, and turnover of the unstable NOTCH intracellular domains is also mediated by FBXW7 [183, 184]. In addition, the RING finger E3 Ubiquitin ligase BC067077/MDM2 (E3 Ub ligase mouse double minute 2), present in the darkmagenta module (BC067077, **Figure 1**), is an oncoprotein in its own right and a negative regulator of p53 protein expression [185].

MYC proteins are regulated by at least five different E3 ubiquitin ligases, including FBXW7 and BTRC [186]. FBXW7 acts as a negative regulator of MYC [187], while BTRC positively regulates MYC protein stability [188]. In addition to control of MYC protein by the UPS, a number of other modulators of MYC activity have prominent positions in key modules. The

STK38 kinase (pink module, **Figure 5**) (upregulated 1.69x in L1-silenced versus controls) regulates MYC protein stability and turnover in a kinase activity-dependent manner. In human B-cell lymphomas, STK38 kinase inactivation prevents apoptosis following B-cell receptor activation, whereas silencing of STK38 decreases MYC levels and promotes apoptosis [189]. STK38 knockdown also suppresses growth of MYC-addicted tumors in vivo [189]. CSNK2A2 (a hub gene in the orange module; **Figure 8**) (fold change -1.76x downregulated in L1-silenced versus controls) also phosphorylates and regulates MYC in addition to multiple transcription factors and Hsp90 and its co-chaperones and regulates Wnt signaling by phosphorylating CTNNB1 [190, 191].

Other oncoproteins targeted by E3 ubiquitin ligases in the modules described here include p53 and NFKBIB/I κ KB (with NFKBIB/I κ KB being present in the darkmagenta module, **Figure 1**). The p53 transcription factor is a tightly regulated sensor of cellular stress and its activation can lead to cell cycle arrest, apoptosis, senescence, DNA repair, altered metabolism, or autophagy [192]. Under normal conditions, protein levels of p53 are kept low by proteasomal degradation, promoted in part through continuous targeting by MDM2 [185]. The transcription of MDM2 is also upregulated by p53, creating a feedback loop in which MDM2 targets both p53 and itself for proteasomal degradation [193]. MDM2 also blocks the transactivating activity of p53, preventing transcriptional activation of p53 target genes [194]. In addition, MDM2 can heterodimerize with the homologous RING finger protein MDM4/MDMX (a hub in the tan module, **Figure 6**). MDM4 binds p53 although it has no intrinsic ubiquitin ligase activity [195]. MDM2 can either mono-ubiquitinate p53, facilitating its transport to the cytoplasm and terminating p53's nuclear activity, or cooperate with MDM4 and other Ub ligases to poly-ubiquitinate and thereby target p53 for degradation by proteasomes [196].

In unstimulated cells, NF κ B proteins are generally kept inactive by binding to proteins known as inhibitors of NF κ B (I κ Bs) [197]. In addition to its actions described above, BTRC triggers ubiquitination of the NF κ B inhibitor, I κ BA [198], with the closely related NFKBIB/I κ B β being a member of the darkmagenta module (**Figure 1**). NF κ B signaling controls many cellular functions, including cell growth and survival, differentiation, development, immunity, and inflammation [199], and is subject to tight post-translational regulation by protein kinases, deubiquitinating enzymes [200], and ubiquitin ligases.

Phosphorylation of I κ BA by IKK targets it for ubiquitination and proteasomal degradation by BTRC, allowing the NF κ B protein, RelA, to translocate to the nucleus and activate gene expression. BTRC also contributes to NF κ B pathway activation by promoting the formation of specific NF κ B protein complexes in the nucleus through ubiquitination and partial proteolysis of I κ Bs, such as p105 and p100. Furthermore, FBXW7 also targets p100 for degradation in a GSK3 β -dependent manner [201–203].

3.15. LINE-1 may affect MYC mRNA stability via MYC's coding region instability determinant

MYC is also subject to regulation at the transcript level. In the mouse, the IGF2BP1 RNA-binding protein stabilizes c-myc RNA by associating with a coding region instability

determinant (CRD) located in the last 249 nucleotides of the coding region of c-myc [204]. Four RNA-binding proteins present in the LINE-1 ORF1p interactome (HNRNPU, SYNCRIP, YBX1, and DHX9) associate with IGF2BP1 in an RNA-dependent fashion and are essential to ensure stabilization of MYC mRNA via its CRD [205]. Complex formation at the CRD may limit transfer of MYC mRNA to polysomes and subsequent translation-coupled decay. Furthermore, IGF2BP2-3, two members of the LINE-1 ORF1p interactome appear to operate redundantly with IGF2BP1 in regulating MYC mRNA in addition to having important roles in modulating tumor cell fate [206].

In further evidence of links between the L1 ORF1p and the IGF2BP1 protein interactomes, Weidensdorfer et al. [205] identified 24 proteins associating with IGF2BP1 by immunoprecipitation and mass spectrometry, 14 of which are also present in the L1 ORF1p interactome. This degree of overlap is highly significant with a representation factor of 115.5 and p -value $< 4.927E-27$.

4. Conclusions

The findings from our WGCNA analysis of the L1-silenced transcriptome in T47D breast cancer cells add weight to the growing body of evidence that L1 expression and activity is a cause rather than a consequence of oncogenesis. In our WGCNA analysis, the observed changes in expression of numerous genes with fundamental roles in cancer and the formation of cancer stem cells or the phenotypic transitions of the EMT/MET seem too concerted and related by function for L1 to be dismissed as a passenger gene or epiphenomenon. Furthermore, a number of these changes in gene expression are consistent with the changes in cancer cell morphology observed upon pharmacological blockade of L1-RT. Our results also support a central hypothesis of the WGCNA method; that the similar expression profiles of genes in a module reflect common regulatory mechanisms or biological functions.

In addition to our gene expression profiling of L1-silencing, we present evidence from independent studies showing statistically significant overlaps between the L1 ORF1p and ORF2p interactomes and cancer driver genes identified by proteomics and data mining. This alone is strongly suggestive of a driver role for L1 in cancer. We also present evidence from independent proteomics studies consistent with L1 having a role in the stabilization of MYC, an oncoprotein with a key role in the global metabolic reprogramming that occurs in cancer.

In summary, we find evidence of L1 activity mounting a concerted attack on cancer cell gene expression consistent with EMT/MET-related phenotypic transitions. L1 activity is also important in the formation of breast cancer stem cells, the support of cancer cell evolvability and, probably, the development of chemoresistance.

Future directions include a more intensive transcriptomic investigation of the effects of L1 on the formation of cancer stem cells with a wider range of cancer cell types and larger sample sizes. Another high priority will be further investigation of the effects of L1 on non-coding RNA and integrating this with the effects seen here on gene expression. In this context, we

have already shown global upregulation of microRNA expression mainly due to a marked increase in let-7 expression following L1-silencing by siRNA [207]. This is consistent with the effects of PANK2 downregulation on Dicer described earlier. It is also likely that the effects of L1-silencing by siRNA differ from those induced by pharmacological blockade of L1-RT and these will need to be investigated to establish whether the concept of pharmacological blockade of L1-RT is therapeutically viable. Chemotherapy is implicated in the formation of drug-resistant cancer stem cells, and NNRTI drugs like Efavirenz are probably no exception to this issue. Finally, thought should be given to targeting the L1 ORF1 protein pharmacologically as it is likely that this has a more important role than L1-RT.

Abbreviations

APOC1	Apolipoprotein C1
BLOC1S1	Biogenesis of lysosomal organelles complex 1 subunit 1
BMP	Bone morphogenetic protein
CML	Chronic myeloid leukemia
CoA	Coenzyme A
CSCs	Cancer stem cells
DAG	Diacylglycerol
DEPTOR	DEP domain containing mTOR-interacting protein
DMSO	Dimethyl sulfoxide
dPANK	Drosophila PANK homolog
eEF1	Eukaryotic translation elongation 1
EIF2S1/eIF2 α	Eukaryotic initiation factor 2
EMT	Epithelial-mesenchymal transition
ESCs	Embryonic stem cells
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit
FKBP6	FK506 binding protein 6
G3BP2	GTPase-activating protein (SH3 domain)-binding protein 2
GBM	Glioblastoma
GSN	Gelsolin
HAF	Hypoxia-associated factor
HERV	Human endogenous retrovirus

HIF-1	Hypoxia inducible factor-1
HSP90	Heat shock protein 90
hTERT	Human telomerase reverse transcriptase
I-DIRT	Isotopic Differentiation of Interactions as Random or Targeted
IkBs	Inhibitors of NFkB
iPSC	Induced pluripotent stem cell
IQGAP1-WASH-exocyst complex:	
IQGAP1	IQ Motif Containing GTPase Activating Protein 1
WASH	Arp2/3 activating protein localized at surface of endosomes where it induces formation of branched actin networks
Exocyst	Octameric protein complex involved in vesicle trafficking and cell migration
L1-KD	L1 knockdown
L1 ORF1p	L1 ORF1 protein
LIMK2	LIM Domain Kinase 2
L1	LINE-1
L1-RT	LINE-1 reverse transcriptase
MET	Mesenchymal-epithelial transition
Miwi2	Mouse homolog of PIWIL4 (Piwi Like RNA-Mediated Gene Silencing 4)
MMP-2	Matrix metalloproteinase 2
MT	Metallothionein
MT1M	Metallothionein 1 M
MT1-MMP	Membrane type 1 metalloprotease
MTF1	Metal-responsive transcription factor
NO	Nitric oxide
NPC	Nuclear pore complex
PA	Phosphatidic acid
PANK2	Pantothenate kinase 2
PDH	Pyruvate dehydrogenase

Pin1	Peptidyl prolyl isomerase 1
PINK1	PTEN-induced putative kinase 1
piRNA	Piwi-interacting RNA
Piwi	P-element Induced Wimpy testis (a subfamily of Argonaute proteins)
PKR	Protein Kinase R
RMA	Robust multi-array average
ROS	Reactive oxygen species
RT	Reverse transcriptase
SART3	Squamous cell carcinoma antigen recognized by T cells 3
SDF-1 α , or CXCL12	Stromal cell-derived factor-1 α
SINEs	Short interspersed elements
SMOH	Smoothened
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SRP	Signal recognition particle
TET	Ten–eleven translocation
TIC	Tumor-initiating cell
TOMM7	Translocase of Outer Mitochondrial Membrane 7
UPS	Ubiquitin–proteasome system
WGCNA	Weighted gene correlation network analysis

Author details

Stephen Ohms^{1*}, Jane E. Dahlstrom² and Danny Rangasamy¹

*Address all correspondence to: stephen.ohms@anu.edu.au

1 John Curtin School of Medical Research, The Australian National University, Canberra, Australia

2 Department of Anatomical Pathology, The Canberra Hospital and ANU Medical School, Garran, Australia

References

- [1] Belancio VP, Hedges DJ, Deininger P. Mammalian non-LTR retrotransposons: For better or worse, in sickness and in health. *Genome Research*. 2008;**18**(3):343-358
- [2] Cordaux R, Batzer MA. The impact of retrotransposons on human genome evolution. *Nature Reviews Genetics*. 2009;**10**(10):691-703
- [3] Beck CR, Collier P, Macfarlane C, Malig M, Kidd JM, Eichler EE, et al. LINE-1 retrotransposition activity in human genomes. *Cell*. 2010;**141**(7):1159-1170
- [4] Brouha B, Schustak J, Badge RM, Lutz-Prigge S, Farley AH, Moran JV, et al. Hot L1s account for the bulk of retrotransposition in the human population. *Proceedings of the National Academy of Sciences*. 2003;**100**(9):5280-5285
- [5] Han JS, Szak ST, Boeke JD. Transcriptional disruption by the L1 retrotransposon and implications for mammalian transcriptomes. *Nature*. 2004;**429**(6989):268-274
- [6] Han JS, Boeke JD. LINE-1 retrotransposons: Modulators of quantity and quality of mammalian gene expression? *BioEssays*. 2005;**27**(8):775-784
- [7] Speek M. Antisense promoter of human L1 retrotransposon drives transcription of adjacent cellular genes. *Molecular and Cellular Biology*. 2001;**21**(6):1973-1985
- [8] Asch H, Eliacin E, Fanning T, Connolly J, Bratthauer G, Asch B. Comparative expression of the LINE-1 p40 protein in human breast carcinomas and normal breast tissues. *Oncology Research*. 1996;**8**(6):239-247
- [9] Harris CR, Normart R, Yang Q, Stevenson E, Haffty BG, Ganesan S, et al. Association of nuclear localization of a long interspersed nuclear element-1 protein in breast tumors with poor prognostic outcomes. *Genes & Cancer*. 2010;**1**(2):115-124
- [10] Ting DT, Lipson D, Paul S, Brannigan BW, Akhavanfard S, Coffman EJ, et al. Aberrant overexpression of satellite repeats in pancreatic and other epithelial cancers. *Science*. 2011;**331**(6017):593-596
- [11] Patnala R, Lee S-H, Dahlstrom JE, Ohms S, Chen L, Dheen ST, et al. Inhibition of LINE-1 retrotransposon-encoded reverse transcriptase modulates the expression of cell differentiation genes in breast cancer cells. *Breast Cancer Research and Treatment*. 2014;**143**(2):239-253
- [12] Bollati V, Fabris S, Pegoraro V, Ronchetti D, Mosca L, Deliliers GL, et al. Differential repetitive DNA methylation in multiple myeloma molecular subgroups. *Carcinogenesis*. 2009;**30**(8):1330-1335
- [13] Fabris S, Bollati V, Agnelli L, Morabito F, Motta V, Cutrona G, et al. Biological and clinical relevance of quantitative global methylation of repetitive DNA sequences in chronic lymphocytic leukemia. *Epigenetics*. 2011;**6**(2):188-194
- [14] Burns KH. Transposable elements in cancer. *Nature Reviews Cancer*. Jul 2017;**17**(7):415-424

- [15] Scott EC, Devine SE. The role of somatic L1 retrotransposition in human cancers. *Viruses*. May 31, 2017;**9**(6). pii: E131
- [16] Yamamura K, Kosumi K, Baba Y, Harada K, Gao F, Zhang X, et al. LINE-1 methylation level and prognosis in pancreas cancer: pyrosequencing technology and literature review. *Surgery Today*. Dec 2017;**47**(12):1450-1459
- [17] Min J, Choi B, Han T-S, Lee H-J, Kong S-H, Suh Y-S, et al. Methylation Levels of LINE-1 as a useful marker for venous invasion in both FFPE and frozen tumor tissues of gastric cancer. *Molecules and Cells*. May 31, 2017;**40**(5):346-354
- [18] Ye ZJ, Liu QP, Cen S, Li XY. The function of LINE-1-encoded reverse transcriptase in tumorigenesis. *Yi Chuan*. May 20, 2017;**39**(5):368-376
- [19] Ardeljan D, Taylor MS, Ting DT, Burns KH. The human long interspersed element-1 retrotransposon: An emerging biomarker of neoplasia. *Clinical Chemistry*. Apr 2017;**63**(4):816-822
- [20] Honda T. Links between human LINE-1 retrotransposons and hepatitis virus-related hepatocellular carcinoma. *Frontiers in Chemistry*. May 11, 2016;**4**:21
- [21] Liu Q, Wang JH, Li XY, Cen S. The connection between LINE-1 retrotransposition and human tumorigenesis. *Yi Chuan*. Feb 2016;**38**(2):93-102
- [22] Sciamanna I, De Luca C, Spadafora C. The reverse transcriptase encoded by LINE-1 retrotransposons in the genesis, progression, and therapy of cancer. *Frontiers in Chemistry*. Feb 11, 2016;**4**:6
- [23] Kemp JR, Longworth MS. Crossing the LINE toward genomic instability: LINE-1 retrotransposition in cancer. *Frontiers in Chemistry*. Dec 16, 2015;**3**:68
- [24] Xiao-Jie L, Hui-Ying X, Qi X, Jiang X, Shi-Jie M. LINE-1 in cancer: Multifaceted functions and potential clinical implications. *Genetics in Medicine*. May 2016;**18**(5):431-439
- [25] Sciamanna I, Gualtieri A, Piazza PF, Spadafora C. Regulatory roles of LINE-1-encoded reverse transcriptase in cancer onset and progression. *Oncotarget*. Sep 30, 2014;**5**(18):8039-8051
- [26] Barchitta M, Quattrocchi A, Maugeri A, Vinciguerra M, Agodi A. LINE-1 hypomethylation in blood and tissue samples as an epigenetic marker for cancer risk: A systematic review and meta-analysis. *PLoS One*. Oct 2, 2014;**9**(10):e109478
- [27] Carreira PE, Richardson SR, Faulkner GJ. L1 retrotransposons, cancer stem cells and oncogenesis. *FEBS Journal*. Jan 2014;**281**(1):63-73
- [28] Baba Y, Murata A, Watanabe M, Baba H. Clinical implications of the LINE-1 methylation levels in patients with gastrointestinal cancer. *Surgery Today*. Oct 2014;**44**(10):1807-1816
- [29] Rodic N, Burns KH. Long interspersed element-1 (LINE-1): Passenger or driver in human neoplasms? *PLoS Genetics*. Mar 2013;**9**(3):e1003402

- [30] Piskareva O, Lackington W, Lemass D, Hendrick C, Doolan P, Barron N. The human L1 element: A potential biomarker in cancer prognosis, current status and future directions. *Current Molecular Medicine*. Jun 2011;**11**(4):286-303
- [31] Montoya-Durango DE, Ramos KS. L1 retrotransposon and retinoblastoma: Molecular linkages between epigenetics and cancer. *Current Molecular Medicine*. Jul 2010;**10**(5): 511-521
- [32] Sinibaldi-Vallebona P, Lavia P, Garaci E, Spadafora C. A role for endogenous reverse transcriptase in tumorigenesis and as a target in differentiating cancer therapy. *Genes, Chromosomes and Cancer*. Jan 2006;**45**(1):1-10
- [33] Aschacher T, Wolf B, Enzmann F, Kienzl P, Messner B, Sampl S, et al. LINE-1 induces hTERT and ensures telomere maintenance in tumour cell lines. *Oncogene*. 2016;**35**(1):94-104
- [34] Cook PR, Jones CE, Furano AV. Phosphorylation of ORF1p is required for L1 retrotransposition. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;**112**(14):4298-4303
- [35] Sciamanna I, Landriscina M, Pittoggi C, Quirino M, Mearelli C, Beraldi R, et al. Inhibition of endogenous reverse transcriptase antagonizes human tumor growth. *Oncogene*. 2005;**24**(24):3923-3931
- [36] Oricchio E, Sciamanna I, Beraldi R, Tolstonog GV, Schumann GG, Spadafora C. Distinct roles for LINE-1 and HERV-K retroelements in cell proliferation, differentiation and tumor progression. *Oncogene*. 2007;**26**(29):4226-4233
- [37] Horvath S, Zhang B, Carlson M, Lu KV, Zhu S, Felciano RM, et al. Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. *Proceedings of the National Academy of Sciences*. 2006;**103**(46):17402-17407
- [38] Lehner B, Crombie C, Tischler J, Fortunato A, Fraser AG. Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways. *Nature Genetics*. 2006;**38**(8):896-903
- [39] Langfelder P, Horvath S. WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*. 2008;**9**(1):559
- [40] R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014. Available from: <http://www.R-project.org/>
- [41] Zhu X, Wang F, Zhao Y, Yang P, Chen J, Sun H, et al. A gain-of-function mutation in Tnni2 impeded bone development through increasing Hif3a expression in DA2B mice. *PLoS Genetics*. 2014;**10**(10):e1004589
- [42] Sidera K, Gaitanou M, Stellas D, Matsas R, Patsavoudi E. A critical role for HSP90 in cancer cell invasion involves interaction with the extracellular domain of HER-2. *Journal of Biological Chemistry*. 2007;**283**(4):2031-2041

- [43] Taylor M, LaCava J, Mita P, Molloy K, Huang C, Li D, et al. Affinity proteomics reveals human host factors implicated in discrete stages of LINE-1 retrotransposition. *Cell*. 2013;**155**(5):1034-1048
- [44] McDonald E, Workman P, Jones K. Inhibitors of the HSP90 molecular chaperone: Attacking the master regulator in cancer. *Current Topics in Medicinal Chemistry*. 2006;**6**(11):1091-1107
- [45] Citri A, Kochupurakkal BS, Yarden Y. The Achilles heel of ErbB-2/HER2: Regulation by the Hsp90 chaperone machine and potential for pharmacological intervention. *Cell Cycle*. 2004;**3**(1):50-59
- [46] Eustace BK, Jay DG. Extracellular roles for the molecular chaperone, HSP90. *Cell Cycle*. 2004;**3**(9):1096-1098
- [47] Nolan KD, Franco OE, Hance MW, Hayward SW, Isaacs JS. Tumor-secreted Hsp90 subverts polycomb function to drive prostate tumor growth and invasion. *The Journal of Biological Chemistry*. 2015;**290**:8271-8282
- [48] Bradley E, Bieberich E, Mivechi N, Tangpisuthipongsa D, Wang G. Regulation of embryonic stem cell pluripotency by heat shock protein 90. *Stem Cells*. 2012;**30**(8):1624-1633
- [49] Jarosz D. Hsp90: A global regulator of the genotype-to-phenotype map in cancers. *Advances in Cancer Research*. 2016;**129**:225-247
- [50] Piacentini L, Fanti L, Specchia V, Bozzetti MP, Berloco M, Palumbo G, Pimpinelli S. Transposons, environmental changes, and heritable induced phenotypic variability. *Chromosoma*. 2014;**123**(4):345-354
- [51] Specchia V, Piacentini L, Tritto P, Fanti L, D'Alessandro R, Palumbo G, Pimpinelli S, Bozzetti MP. Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. *Nature*. 2010;**463**:662-665
- [52] Xiol J, Cora E, Kogelgruber R, Chuma S, Subramanian S, Hosokawa M, et al. A role for Fkbp6 and the chaperone machinery in piRNA amplification and transposon silencing. *Molecular Cell*. 2012;**47**(6):970-979
- [53] Goodier JL, Cheung LE, Kazazian HH. Mapping the LINE1 ORF1 protein interactome reveals associated inhibitors of human retrotransposition. *Nucleic Acids Research*. 2013;**41**(15):7401-7419
- [54] Chang C, Hautbergue G, Walsh M, Viphakone N, van Dijk T, Philipsen S, et al. Chtop is a component of the dynamic TREX mRNA export complex. *The EMBO Journal*. 2013;**32**(3):473-486
- [55] Luna R, Rondón AG, Aguilera A. New clues to understand the role of THO and other functionally related factors in mRNP biogenesis. *Biochimica et Biophysica Acta (BBA) – Gene Regulatory Mechanisms*. 2012;**1819**(6):514-520

- [56] Viphakone N, Hautbergue GM, Walsh M, Chang C, Holland A, Folco EG, et al. TREX exposes the RNA-binding domain of Nxf1 to enable mRNA export. *Nature Communications*. 2012;**3**:1006
- [57] Müller-McNicoll M, Botti V, de Jesus Domingues AM, Brandl H, Schwich OD, Steiner MC, et al. SR proteins are NXF1 adaptors that link alternative RNA processing to mRNA export. *Genes & Development*. 2016;**30**(5):553-566
- [58] Dufu K, Livingstone MJ, Seebacher J, Gygi SP, Wilson SA, Reed R. ATP is required for interactions between UAP56 and two conserved mRNA export proteins, Aly and CIP29, to assemble the TREX complex. *Genes & Development*. 2010;**24**(18):2043-2053
- [59] Lindtner S, Felber B, Kjems J. An element in the 3' untranslated region of human LINE-1 retrotransposon mRNA binds NXF1(TAP) and can function as a nuclear export element. *RNA*. 2002;**8**(3):345-356
- [60] Kato M, Wei M, Yamano S, Kakehashi A, Tamada S, Nakatani T, et al. DDX39 acts as a suppressor of invasion for bladder cancer. *Cancer Science*. 2012;**103**(7):1363-1369
- [61] Park H, Imoto S, Miyano S. Recursive random lasso (RRLasso) for identifying anti-cancer drug targets. *PLoS One*. 2015;**10**(11):e0141869
- [62] Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Research*. 2017;**45**(Database issue):D362-D3D8
- [63] Siudeja K, Srinivasan B, Xu L, Rana A, de Jong J, Nollen E, et al. Impaired Coenzyme A metabolism affects histone and tubulin acetylation in *Drosophila* and human cell models of pantothenate kinase associated neurodegeneration. *EMBO Molecular Medicine*. 2011;**3**(12):755-766
- [64] Wang W, Eddy R, Condeelis J. The cofilin pathway in breast cancer invasion and metastasis. *Nature Reviews. Cancer*. 2007;**7**(6):429-440
- [65] Ward DM, Kaplan J. Ferroportin-mediated iron transport: Expression and regulation. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research*. 2012;**1823**(9):1426-1433
- [66] Poli M, Derosas M, Luscieti S, Cavadini P, Campanella A, Verardi R, et al. Pantothenate kinase-2 (Pank2) silencing causes cell growth reduction, cell-specific ferroportin up-regulation and iron deregulation. *Neurobiology of Disease*. 2010;**39**(2):204-210
- [67] Mai TT, Hamai A, Hienzsch A, Cañeque T, Müller S, Wicinski J, et al. Salinomycin kills cancer stem cells by sequestering iron in lysosomes. *Nature Chemistry*. 2017;**9**:1025-1033
- [68] Pinnix ZK, Miller LD, Wang W, D'Agostino R, Kute T, Willingham MC, et al. Ferroportin and iron regulation in breast cancer progression and prognosis. *Science Translational Medicine*. 2010;**2**(43):43ra56-43ra56

- [69] Yamane K, Tateishi K, Klose RJ, Fang J, Fabrizio LA, Erdjument-Bromage H, et al. PLU-1 is an H3K4 demethylase involved in transcriptional repression and breast cancer cell proliferation. *Molecular Cell*. 2007;**25**(6):801-812
- [70] Yamamoto S, Wu Z, Russnes HG, Takagi S, Peluffo G, Vaske C, et al. JARID1B is a luminal lineage-driving oncogene in breast cancer. *Cancer Cell*. 2014;**25**(6):762-777
- [71] Greer EL, Shi Y. Histone methylation: A dynamic mark in health, disease and inheritance. *Nature Reviews Genetics*. 2012;**13**(5):343-357
- [72] Shen L, Wu H, Diep D, Yamaguchi S, D'Alessio AC, Fung H-L, et al. Genome-wide analysis reveals TET- and TDG-dependent 5-methylcytosine oxidation dynamics. *Cell*. 2013;**153**(3):692-706
- [73] Tsai Y-P, Chen H-F, Chen S-Y, Cheng W-C, Wang H-W, Shen Z-J, et al. TET1 regulates hypoxia-induced epithelial-mesenchymal transition by acting as a co-activator. *Genome Biology*. 2014 Dec 3;**15**(12):513. DOI: 10.1186/s13059-014-0513-0
- [74] Hu X, Zhang L, Mao S-Q, Li Z, Chen J, Zhang R-R, et al. Tet and TDG mediate DNA demethylation essential for mesenchymal-to-epithelial transition in somatic cell reprogramming. *Cell Stem Cell*. 2014;**14**(4):512-522
- [75] Schonberg DL, Miller TE, Wu Q, Flavahan WA, Das NK, Hale JS, et al. Preferential iron trafficking characterizes glioblastoma stem-like cells. *Cancer Cell*. 2015;**28**(4):441-455
- [76] Lerman MI, Minna JD. The 630-kb lung cancer homozygous deletion region on human chromosome 3p21.3: Identification and evaluation of the resident candidate tumor suppressor genes. *Cancer Research*. 2000;**60**(21):6116
- [77] Ji L, Nishizaki M, Gao B, Burbee D, Kondo M, Kamibayashi C, et al. Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities in vitro and in vivo. *Cancer Research*. 2002;**62**(9):2715-2720
- [78] Arber C, Angelova PR, Wiethoff S, Tsuchiya Y, Mazzacuva F, Preza E, et al. iPSC-derived neuronal models of PANK2-associated neurodegeneration reveal mitochondrial dysfunction contributing to early disease. *PLoS One*. 2017;**12**(9):e0184104
- [79] Dansie LE, Reeves S, Miller K, Zano SP, Frank M, Pate C, et al. Physiological roles of the pantothenate kinases. *Biochemical Society transactions*. 2014;**42**(4):1033-1036
- [80] Garcia M, Leonardi R, Zhang Y-M, Rehg JE, Jackowski S. Germline deletion of pantothenate kinases 1 and 2 reveals the key roles for CoA in postnatal metabolism. *PLoS One*. 2012;**7**(7):e40871
- [81] Ding S, Li C, Cheng N, Cui X, Xu X, Zhou G. Redox regulation in cancer stem cells. *Oxidative Medicine and Cellular Longevity*. 2015;**2015**:750798
- [82] Babula P, Masarik M, Adam V, Eckschlager T, Stiborova M, Trnkova L, et al. Mammalian metallothioneins: Properties and functions. *Metallomics*. 2012;**4**(8):739

- [83] Coyle P, Mathew G, Game PA, Myers JC, Philcox JC, Rofe AM, et al. Metallothionein in human oesophagus, Barrett's epithelium and adenocarcinoma. *British Journal of Cancer*. 2002;**87**(5):533-536
- [84] Palmiter RD. The elusive function of metallothioneins. *Proceedings of the National Academy of Sciences*. 1998;**95**(15):8428-8430
- [85] Gumulec J, Raudenska M, Adam V, Kizek R, Masarik M. Metallothionein – Immunohistochemical cancer biomarker: A meta-analysis. *PLoS One*. 2014;**9**(1)
- [86] Murphy BJ, Kimura T, Sato BG, Shi Y, Andrews GK. Metallothionein induction by hypoxia involves cooperative interactions between metal-responsive transcription factor-1 and hypoxia-inducible transcription factor-1. *Molecular Cancer Research*. 2008;**6**(3):483-490
- [87] Dubé A, Harrisson J, Saint-Gelais G, Séguin C. Hypoxia acts through multiple signaling pathways to induce metallothionein transactivation by the metal-responsive transcription factor-1 (MTF-1). *Biochemistry and Cell Biology*. 2011;**89**(6):562-577
- [88] Feng W, Wang Y, Cai L, Kang YJ. Metallothionein rescues hypoxia-inducible factor-1 transcriptional activity in cardiomyocytes under diabetic conditions. *Biochemical and Biophysical Research Communications*. 2007;**360**(1):286-289
- [89] Kojima I, Tanaka T, Inagi R, Nishi H, Aburatani H, Kato H, et al. Metallothionein is upregulated by hypoxia and stabilizes hypoxia-inducible factor in the kidney. *Kidney International*. 2009;**75**(3):268-277
- [90] Troadec M, Ward D, Lo E, Kaplan J, De Domenico I. Induction of FPN1 transcription by MTF-1 reveals a role for ferroportin in transition metal efflux. *Blood*. 2010;**116**(22):4657-4664
- [91] Huang C, Yang W, Chang S, Tai S, Tzeng C, Kao J, et al. Regulation of membrane-type 4 matrix metalloproteinase by SLUG contributes to hypoxia-mediated metastasis. *Neoplasia*. 2009;**11**(12):1371-IN14
- [92] Storci G, Sansone P, Mari S, D'Uva G, Tavolari S, Guarneri T, et al. TNFalpha up-regulates SLUG via the NF-kappaB/HIF1alpha axis, which imparts breast cancer cells with a stem cell-like phenotype. *Journal of Cellular Physiology*. 2010;**225**(3):682-691
- [93] Alves CC, Carneiro, F, Hoefler H, Becker KF. Role of the epithelial-mesenchymal transition regulator Slug in primary human cancers. *Frontiers in Bioscience*. 2009;(14):3035-3050
- [94] Storci G, Sansone P, Trere D, Tavolari S, Taffurelli M, Ceccarelli C, et al. The basal-like breast carcinoma phenotype is regulated by SLUG gene expression. *The Journal of Pathology*. 2008;**214**(1):25-37
- [95] Zhang J, Zhang F, Hong C, Giuliano A, Cui X, Zhou G, et al. Critical protein GAPDH and its regulatory mechanisms in cancer cells. *Cancer Biology & Medicine*. 2015;**12**(1):10-22

- [96] Nicholls C, Pinto AR, Li H, Li L, Wang L, Simpson R, et al. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) induces cancer cell senescence by interacting with telomerase RNA component. *Proceedings of the National Academy of Sciences*. 2012;**109**(33):13308-13313
- [97] David L, Polo JM. Phases of reprogramming. *Stem Cell Research*. 2014;**12**(3):754-761
- [98] Sakurai K, Talukdar I, Patil VS, Dang J, Li Z, Chang K, et al. Kinome-wide functional analysis highlights the role of cytoskeletal remodeling in somatic cell reprogramming. *Cell Stem Cell*. 2014;**14**(4):523-534
- [99] Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, et al. Systematic variation in gene expression patterns in human cancer cell lines. *Nature Genetics*. 2000;**24**(3):227-235
- [100] Lightfoot J, Hitzler JK, Zipursky A, Albert M, Macgregor PF. Distinct gene signatures of transient and acute megakaryoblastic leukemia in down syndrome. *Leukemia*. 2004;**18**(10):1617-1623
- [101] Takano S, Yoshitomi H, Togawa A, Sogawa K, Shida T, Kimura F, et al. Apolipoprotein C-1 maintains cell survival by preventing from apoptosis in pancreatic cancer cells. *Oncogene*. 2007;**27**(20):2810-2822
- [102] Ko H, Wang Y, Fong W, Chi M, Chi K, Kao S. Apolipoprotein C1 (APOC1) as a novel diagnostic and prognostic biomarker for lung cancer: A marker phase I trial. *Thoracic Cancer*. 2014;**5**(6):500-508
- [103] Nishimura K, Aizawa S, Nugroho FL, Shiomitsu E, Tran YTH, Bui PL, et al. A role for KLF4 in promoting the metabolic shift via TCL1 during induced pluripotent stem cell generation. *Stem Cell Reports*. 2017;**8**(3):787-801
- [104] Baskerville S, Bartel DP. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. *RNA*. 2005;**11**(3):241-247
- [105] Wilfred BR, Wang W-X, Nelson PT. Energizing miRNA research: A review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. *Molecular Genetics and Metabolism*. 2007;**91**(3):209-217
- [106] Martello G, Rosato A, Ferrari F, Manfrin A, Cordenonsi M, Dupont S, et al. A MicroRNA targeting dicer for metastasis control. *Cell*. 2010;**141**(7):1195-1207
- [107] Vazquez-Martin A, den Haute C, Cufí S, Faja B, Cuyàs E, Lopez-Bonet E, et al. Mitophagy-driven mitochondrial rejuvenation regulates stem cell fate. *Aging*. 2016;**8**(7):1330-1352
- [108] Hasson SA, Kane LA, Yamano K, Huang C-H, Sliter DA, Buehler E, et al. High-content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy. *Nature*. 2013;**504**(7479):291-295
- [109] Requejo-Aguilar R, Lopez-Fabuel I, Fernandez E, Martins LM, Almeida A, Bolaños JP. PINK1 deficiency sustains cell proliferation by reprogramming glucose metabolism through HIF1. *Nature Communications*. 2014;**5**:4514

- [110] Molloy KR, Taylor MS, Altukhov I, Mita P, Jiang H, Adney EM, et al. Dissection of purified LINE-1 reveals distinct nuclear and cytoplasmic intermediates. *bioRxiv* 157818, cold Spring Harbor. The Laboratory. 2017
- [111] Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial–mesenchymal transition. *Nature reviews Molecular cell biology*. 2014;**15**(3):178-196
- [112] Zhuo J, Tan EH, Yan B, Tothhawng L, Jayapal M, Koh S, et al. Gelsolin induces colorectal tumor cell invasion via modulation of the Urokinase-type plasminogen activator Cascade. *PLoS One*. 2012;**7**(8):e43594
- [113] Monteiro P, Rossé C, Castro-Castro A, Irondelle M, Lagoutte E, Paul-Gilloteaux P, et al. Endosomal WASH and exocyst complexes control exocytosis of MT1-MMP at invadopodia. *The Journal of Cell Biology*. 2013;**203**(6):1063-1079
- [114] Morris HT, Machesky LM. Actin cytoskeletal control during epithelial to mesenchymal transition: Focus on the pancreas and intestinal tract. *British Journal of Cancer*. 2015; **112**(4):613-620
- [115] Duleh SN, Welch MD. WASH and the Arp2/3 complex regulate endosome shape and trafficking. *Cytoskeleton (Hoboken)*. 2010;**67**(3):193-206
- [116] Samavarchi-Tehrani P, Golipour, A, David L, Sung HK, Beyer TA, Datti A, et al. Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell*. 2010;**7**(1):64-77
- [117] Kelly CE, Thymiakou E, Dixon JE, Tanaka S, Godwin J, Episkopou V. Rnf165/Ark2C enhances BMP-Smad signaling to mediate motor axon extension. *PLoS Biology*. 2013; **11**(4):e1001538
- [118] van Cuijk L, van Belle GJ, Turkyilmaz Y, Poulsen SL, Janssens RC, Theil AF, et al. SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair. *Nature Communications*. 2015;**6**:7499
- [119] Sharma V, Antonacopoulou AG, Tanaka S, Panoutsopoulos AA, Bravou V, Kalofonos HP, et al. Enhancement of TGF-signaling responses by the E3 ubiquitin ligase Arkadia provides tumor suppression in colorectal cancer. *Cancer Research*. 2011;**71**(20): 6438-6449
- [120] Briones-Orta MA, Levy L, Madsen CD, Das D, Erker Y, Sahai E, et al. Arkadia regulates tumor metastasis by modulation of the TGF- pathway. *Cancer Research*. 2013;**73**(6): 1800-1810
- [121] Chen H, Yang T, Lei Z, Wang L, Yang H, Tong X, et al. RNF111/Arkadia is regulated by DNA methylation and affects TGF- β /Smad signaling associated invasion in NSCLC cells. *Lung Cancer*. 2015;**90**(1):32-40
- [122] Mizutani A, Koinuma D, Seimiya H, Miyazono K. The Arkadia-ESRP2 axis suppresses tumor progression: Analyses in clear-cell renal cell carcinoma. *Oncogene*. 2016;**35**(27): 3514-3523

- [123] Xu A, Sun S. Genomic profiling screens small molecules of metastatic prostate carcinoma. *Oncology Letters*. 2015 Sep;**10**(3):1402-1408
- [124] Ullu E, Tschudi C. Alu sequences are processed 7SL RNA genes. *Nature*. 1984;**312**(5990):171-172
- [125] Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J. Initial sequencing and analysis of the human genome. *Nature*. 2001;**409**(6822):860-921
- [126] Hsu K, Chang D, Maraia R. Human signal recognition particle (SRP) Alu-associated protein also binds Alu interspersed repeat sequence RNAs. *Journal of Biological Chemistry*. 1995;**270**(17):10179-10186
- [127] Bennett EA, Keller H, Mills RE, Schmidt S, Moran JV, Weichenrieder O, et al. Active Alu retrotransposons in the human genome. *Genome Research*. 2008;**18**:1875-1883
- [128] Berger A, Ivanova E, Gareau C, Scherrer A, Mazroui R, Strub K. Direct binding of the Alu binding protein dimer SRP9/14 to 40S ribosomal subunits promotes stress granule formation and is regulated by Alu RNA. *Nucleic Acids Research*. 2014;**42**(17):11203-11217
- [129] Gupta N, Badaeux M, Liu Y, Naxerova K, Sgroi D, Munn LL, et al. Stress granule-associated protein G3BP2 regulates breast tumor initiation. *Proceedings of the National Academy of Sciences*. 2017;**114**(5):1033-1038
- [130] Goodier JL, Zhang L, Vetter MR, Kazazian HH. LINE-1 ORF1 protein localizes in stress granules with other RNA-binding proteins, including components of RNA interference RNA-induced silencing complex. *Molecular and Cellular Biology*. 2007;**27**(18):6469-6483
- [131] Liu Y, Timani K, Ou X, Broxmeyer HE, He JJ. C-MYC controlled TIP110 protein expression regulates OCT4 mRNA splicing in human embryonic stem cells. *Stem Cells and Development*. 2013;**22**(5):689-694
- [132] Timani KA, Liu Y, He JJ. Tip110 interacts with YB-1 and regulates each other's function. *BMC Molecular Biology*. 2013;**14**:14
- [133] Koh MY, Darnay BG, Powis G. Hypoxia-associated factor, a novel E3-ubiquitin ligase, binds and ubiquitinates hypoxia-inducible factor 1 α , leading to its oxygen-independent degradation. *Molecular and Cellular Biology*. 2008;**28**(23):7081-7095
- [134] Koh MY, Lemos R, Liu X, Powis G. The hypoxia-associated factor switches cells from HIF-1 α - To HIF-2 α -dependent signaling promoting stem cell characteristics, aggressive tumor growth and invasion. *Cancer Research*. 2011;**71**(11):4015-4027
- [135] Dominguez CL, Floyd DH, Xiao A, Mullins GR, Kefas BA, Xin W, et al. Diacylglycerol kinase alpha is a critical signaling node and novel therapeutic target in glioblastoma and other cancers. *Cancer Discovery*. 2013;**3**(7):782-797
- [136] Purow B. Molecular pathways: Targeting diacylglycerol kinase alpha in cancer. *Clinical Cancer Research*. 2015;**21**(22):5008-5012

- [137] Rainero E, Cianflone C, Porporato PE, Chianale F, Malacarne V, Bettio V, et al. The diacylglycerol kinase α /atypical PKC/ β 1 integrin pathway in SDF-1 α mammary carcinoma invasiveness. *PLoS One*. 2014;**9**(6):e97144
- [138] Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature*. 2001;**410**(6824):50-56
- [139] Korkaya H, Liu S, Wicha MS. Breast cancer stem cells, cytokine networks, and the tumor microenvironment. *The Journal of Clinical Investigation*. 2011;**121**(10):3804-3809
- [140] Teicher BA, Fricker SP. CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clinical Cancer Research*. 2010;**16**(11):2927-2931
- [141] Rainero E, Caswell PT, Muller PA, Grindlay J, McCaffrey MW, Zhang Q, et al. Diacylglycerol kinase α controls RCP-dependent integrin trafficking to promote invasive migration. *The Journal of Cell Biology*. 2012;**196**(2):277-295
- [142] Offermanns S, Simon MI. G α 15 and G α 16 couple a wide variety of receptors to phospholipase C. *Journal of Biological Chemistry*. 1995;**270**(25):15175-15180
- [143] Strasser RH, Benovic JL, Caron MG, Lefkowitz RJ. Beta-agonist- and prostaglandin E1-induced translocation of the beta-adrenergic receptor kinase: Evidence that the kinase may act on multiple adenylate cyclase-coupled receptors. *Proceedings of the National Academy of Sciences*. 1986;**83**(17):6362-6366
- [144] Benovic JL, Strasser RH, Caron MG, Lefkowitz RJ. Beta-adrenergic receptor kinase: Identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. *Proceedings of the National Academy of Sciences*. 1986;**83**(9):2797-2801
- [145] Attramadal H, Arriza JL, Aoki C, Dawson TM, Codina J, Kwatra MM, et al. Beta-arrestin2, a novel member of the arrestin/beta-arrestin gene family. *Journal of Biological Chemistry*. 1992;**267**(25):17882-17890
- [146] Lohse M, Benovic J, Codina J, Caron M, Lefkowitz R. Beta-arrestin: A protein that regulates beta-adrenergic receptor function. *Science*. 1990;**248**(4962):1547-1550
- [147] Giannone F, Malpeli G, Lisi V, Grasso S, Shukla P, Ramarli D, et al. The puzzling uniqueness of the heterotrimeric G15 protein and its potential beyond hematopoiesis. *Journal of Molecular Endocrinology*. 2010;**44**(5):259-269
- [148] Philip PA, Mooney M, Jaffe D, Eckhardt G, Moore M, Meropol N, et al. Consensus report of the National Cancer Institute clinical trials planning meeting on pancreas cancer treatment. *Journal of Clinical Oncology*. 2009;**27**(33):5660-5569
- [149] Waldron RT, Innamorati G, Torres-Marquez ME, Sinnott-Smith J, Rozengurt E. Differential PKC-dependent and -independent PKD activation by G protein α subunits of the Gq family: Selective stimulation of PKD Ser748 autophosphorylation by G α q. *Cellular Signalling*. 2012;**24**(4):914-921

- [150] Harikumar KB, Kunnumakkara AB, Ochi N, Tong Z, Deorukhkar A, Sung B, et al. A novel small molecule inhibitor of protein kinase D blocks pancreatic cancer growth in vitro and in vivo. *Molecular Cancer Therapeutics*. 2010;**9**(5):1136-1146
- [151] Singh S, Srivastava SK, Bhardwaj A, Owen LB, Singh AP. CXCL12–CXCR4 signalling axis confers gemcitabine resistance to pancreatic cancer cells: A novel target for therapy. *British Journal of Cancer*. 2010;**103**(11):1671-1679
- [152] Kayali AG, Van Gunst K, Campbell IL, Stotland A, Kritzik M, Liu G, et al. The stromal cell–derived factor-1 α /CXCR4 ligand–receptor axis is critical for progenitor survival and migration in the pancreas. *The Journal of Cell Biology*. 2003;**163**(4):859-869
- [153] Contos JJA, Ye X, Sah VP, Chun J. Tandem genomic arrangement of a G protein (Gna15) and G protein-coupled receptor (s1p4/lpC1/Edg6) gene. *FEBS Letters*. 2002;**531**(1):99-102
- [154] Inniss K, Moore H. Mediation of apoptosis and proliferation of human embryonic stem cells by Sphingosine-1-phosphate. *Stem Cells and Development*. 2006;**15**(6):789-796
- [155] Serafimidis I, Heximer S, Beis D, Gavalas A. G protein-coupled receptor signaling and Sphingosine-1-phosphate play a Phylogenetically conserved role in endocrine pancreas morphogenesis. *Molecular and Cellular Biology*. 2011;**31**(22):4442-4453
- [156] Morris JP, Wang SC, Hebrok M. KRAS, hedgehog, Wnt and the twisted developmental biology of pancreatic ductal adenocarcinoma. *Nature Reviews Cancer*. 2010;**10**(10):683-695
- [157] Wray J, Hartmann C. WNTing embryonic stem cells. *Trends in Cell Biology*. 2012;**22**(3):159-168
- [158] Masdeu C, Faure H, Coulombe J, Schoenfelder A, Mann A, Brabet I, et al. Identification and characterization of hedgehog modulator properties after functional coupling of smoothed to G15. *Biochemical and Biophysical Research Communications*. 2006;**349**(2):471-479
- [159] Morton JP, Mongeau ME, Klimstra DS, Morris JP, Lee YC, Kawaguchi Y, et al. Sonic hedgehog acts at multiple stages during pancreatic tumorigenesis. *Proceedings of the National Academy of Sciences*. 2007;**104**(12):5103-5108
- [160] Giovinazzo F, Malpeli G, Zanini S, Parenti M, Piemonti L, Colombatti M, et al. Ectopic expression of the heterotrimeric G15 protein in pancreatic carcinoma and its potential in cancer signal transduction. *Cellular Signalling*. 2013;**25**(3):651-659
- [161] de Jonge HJM, Woolthuis CM, Vos AZ, Mulder A, van den Berg E, Kluin PM, et al. Gene expression profiling in the leukemic stem cell-enriched CD34+ fraction identifies target genes that predict prognosis in normal karyotype AML. *Leukemia*. 2011;**25**(12):1825-1833
- [162] Avasarala S, Bikkavilli RK, Van Scoyk M, Zhang W, Lapite A, Hostetter L, et al. Heterotrimeric G-protein, G α 16, is a critical downstream effector of non-canonical Wnt signaling and a potent inhibitor of transformed cell growth in non-small cell lung cancer. *PLoS One*. 2013;**8**(10):e76895

- [163] Hu S, Li J, Xu F, Mei S, Le Duff Y, Yin L, et al. SAMHD1 inhibits LINE-1 retrotransposition by promoting stress granule formation. *PLOS Genetics*. 2015;**11**(7):e1005367
- [164] Irving A, Wang D, Vasilevski O, Latchoumanin O, Kozer N, Clayton A, et al. Regulation of actin dynamics by protein kinase R control of gelsolin enforces basal innate immune defense. *Immunity*. 2012;**36**(5):795-806
- [165] DiDonato JA, Mercurio F, Karin M. NF- κ B and the link between inflammation and cancer. *Immunological Reviews*. 2012;**246**(1):379-400
- [166] Tirinato L, Pagliari F, Limongi T, Marini M, Falqui A, Seco J, et al. An overview of lipid droplets in cancer and cancer stem cells. *Stem Cells International*. 2017;**2017**(2017):1656053
- [167] Pelletier J, Graff J, Ruggero D, Sonenberg N. Targeting the eIF4F translation initiation complex: A critical nexus for cancer development. *Cancer Research*. 2015;**75**(2):250-263
- [168] Mitchell SF, Lorsch JR. Should I stay or should I go? Eukaryotic translation initiation factors 1 and 1A control start codon recognition. *Journal of Biological Chemistry*. 2008;**283**(41):27345-27349
- [169] Joseph P, Lei Y-X, Ong T-m. Up-regulation of expression of translation factors – A novel molecular mechanism for cadmium carcinogenesis. *Molecular and Cellular Biochemistry*. 2004;**255**(1):93-101
- [170] Gonen H, Smith CE, Siegel NR, Kahana C, Merrick WC, Chakraborty K, et al. Protein synthesis elongation factor EF-1 alpha is essential for ubiquitin-dependent degradation of certain N alpha-acetylated proteins and may be substituted for by the bacterial elongation factor EF-Tu. *Proceedings of the National Academy of Sciences of the United States of America*. 1994;**91**(16):7648-7652
- [171] Hotokezaka Y, Többen U, Hotokezaka H, van Leyen K, Beatrix B, Smith DH, et al. Interaction of the eukaryotic elongation factor 1A with newly synthesized polypeptides. *Journal of Biological Chemistry*. 2002;**277**(21):18545-18551
- [172] Ruest L-B, Marcotte R, Wang E. Peptide elongation factor eEF1A-2/S1 expression in cultured differentiated myotubes and its protective effect against Caspase-3-mediated apoptosis. *Journal of Biological Chemistry*. 2002;**277**(7):5418-5425
- [173] Chang R, Wang E. Mouse translation elongation factor eEF1A-2 interacts with Prdx-I to protect cells against apoptotic death induced by oxidative stress. *Journal of Cellular Biochemistry*. 2007;**100**(2):267-278
- [174] Murthi A, Shaheen HH, Huang H-Y, Preston MA, Lai T-P, Phizicky EM, et al. Regulation of tRNA bidirectional nuclear-cytoplasmic trafficking in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell*. 2010;**21**(4):639-649
- [175] Shamovsky I, Ivannikov M, Kandel ES, Gershon D, Nudler E. RNA-mediated response to heat shock in mammalian cells. *Nature*. 2006;**440**(7083):556-560

- [176] Kim S, Coulombe PA. Emerging role for the cytoskeleton as an organizer and regulator of translation. *Nature Reviews. Molecular Cell Biology*. 2010;**11**(1):75-81
- [177] Morrissey C, Schwefel D, Ennis-Adeniran V, Taylor IA, Crow YJ, Webb M. The eukaryotic elongation factor eEF1A1 interacts with SAMHD1. *Biochemical Journal*. 2015;**466**(1):69-76
- [178] Malta-Vacas J, Aires C, Costa P, Conde AR, Ramos S, Martins AP, et al. Differential expression of the eukaryotic release factor 3 (eRF3/GSPT1) according to gastric cancer histological types. *Journal of Clinical Pathology*. 2005;**58**(6):621-625
- [179] Schoenberg DR, Maquat LE. Regulation of cytoplasmic mRNA decay. *Nature Reviews. Genetics* 2012;**13**(4):246-259
- [180] Jerbi S, Jolles B, Bouceba T, Jean-Jean O. Studies on human eRF3-PABP interaction reveal the influence of eRF3a N-terminal glycine repeat on eRF3-PABP binding affinity and the lower affinity of eRF3a 12-GGC allele involved in cancer susceptibility. *RNA Biology*. 2016;**13**(3):306-315
- [181] Mao JH, Kim IJ, Wu D, Climent J, Kang HC, DelRosario R, et al. FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. *Science*. 2008;**321**(5895):1499-1502
- [182] Zhao Y, Sun Y. Targeting the mTOR-DEPTOR pathway by CRL E3 ubiquitin ligases: Therapeutic application. *Neoplasia*. 2012;**14**(5):360-367
- [183] Öberg C, Li J, Pauley A, Wolf E, Gurney M, Lendahl U. The Notch intracellular domain is ubiquitinated and negatively regulated by the mammalian Sel-10 homolog. *Journal of Biological Chemistry*. 2001;**276**(38):35847-35853
- [184] Gupta-Rossi N, Le Bail O, Gonen H, Brou C, Logeat F, Six E, et al. Functional interaction between SEL-10, an F-box protein, and the nuclear form of activated Notch1 receptor. *Journal of Biological Chemistry*. 2001;**276**(37):34371-34378
- [185] Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature*. 1997;**387**(6630):296-299
- [186] Thomas L, Tansey W. Proteolytic Control of the Oncoprotein Transcription Factor Myc. *Advances in Cancer Research*. 2011;**110**:77-106
- [187] Choi SH, Wright JB, Gerber SA, Cole MD. Myc protein is stabilized by suppression of a novel E3 ligase complex in cancer cells. *Genes & Development*. 2010;**24**(12):1236-1241
- [188] Popov N, Schülein C, Jaenicke LA, Eilers M. Ubiquitylation of the amino terminus of Myc by SCF β -TrCP antagonizes SCFFbw7-mediated turnover. *Nature Cell Biology*. 2010;**12**(10):973-981
- [189] Bisikirska BC, Adam SJ, Alvarez MJ, Rajbhandari P, Cox R, Lefebvre C, et al. STK38 is a critical upstream regulator of MYC's oncogenic activity in human B-cell lymphoma. *Oncogene*. 2013;**32**(45):5283-5291

- [190] Song DH, Sussman DJ, Seldin DC. Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. *Journal of Biological Chemistry*. 2000;**275**(31):23790-23797
- [191] Dominguez I, Sonenshein GE, Seldin DC. CK2 and its role in Wnt and NF- κ B signaling: Linking development and cancer. *Cellular and Molecular Life Sciences*. 2009;**66**(11-12):1850-1857
- [192] Vousden KH, Prives C. Blinded by the light: The growing complexity of p53. *Cell*. 2009;**137**(3):413-431
- [193] Fang S, Jensen JP, Ludwig RL, Vousden KH, Weissman AM. Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. *Journal of Biological Chemistry*. 2000;**275**(12):8945-8951
- [194] Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell*. 1992;**69**(7):1237-1245
- [195] Jackson MW, Berberich SJ. MdmX protects p53 from Mdm2-mediated degradation. *Molecular and Cellular Biology*. 2000;**20**(3):1001-1007
- [196] Wang X, Jiang X. Mdm2 and MdmX partner to regulate p53. *FEBS Letters*. 2012;**586**(10):1390-1396
- [197] Gilmore TD. Introduction to NF- κ B: Players, pathways, perspectives. *Oncogene*. 2006;**25**(51):6680-6684
- [198] Karin M. Nuclear factor- κ B in cancer development and progression. *Nature*. 2006;**441**(7092):431-436
- [199] Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF- κ B as the matchmaker. *Nature Immunology*. 2011;**12**(8):715-723
- [200] Harhaj EW, Dixit VM. Deubiquitinases in the regulation of NF- κ B signaling. *Cell Research*. 2011;**21**(1):22-39
- [201] Arabi A, Ullah K, Branca RM, Johansson J, Bandarra D, Haneklaus M, et al. Proteomic screen reveals Fbw7 as a modulator of the NF- κ B pathway. *Nature Communications*. 2012;**3**:976
- [202] Busino L, Millman SE, Scotto L, Kyratsous CA, Basrur V, O'Connor O, et al. Fbxw7 α - and GSK3-mediated degradation of p100 is a pro-survival mechanism in multiple myeloma. *Nature Cell Biology*. 2012;**14**(4):375-385
- [203] Fukushima H, Matsumoto A, Inuzuka H, Zhai B, Lau AW, Wan L, et al. SCF(Fbw7) modulates the NF κ B signaling pathway by targeting NF κ B2 for ubiquitination and destruction. *Cell Reports*. 2012;**1**(5):434-443
- [204] Wisdom R, Lee W. The protein-coding region of c-myc mRNA contains a sequence that specifies rapid mRNA turnover and induction by protein synthesis inhibitors. *Genes & Development*. 1991;**5**(2):232-243

- [205] Weidensdorfer D, Stohr N, Baude A, Lederer M, Kohn M, Schierhorn A, et al. Control of c-myc mRNA stability by IGF2BP1-associated cytoplasmic RNPs. *Ribonucleic Acid*. 2009;**15**(1):104-115
- [206] Lederer M, Bley N, Schleifer C, Hüttelmaier S. The role of the oncofetal IGF2 mRNA-binding protein 3 (IGF2BP3) in cancer. *Seminars in Cancer Biology*. 2014;**29**:3-12
- [207] Ohms S, Rangasamy D. Silencing of LINE-1 retrotransposons contributes to variation in small noncoding RNA expression in human cancer cells. *Oncotarget*. 2014;**5**(12):4103-4117

IntechOpen