SPANDIDOS PUBLICATIONS

ONCOLOGY REPORTS 40: 123-144, 2018

Potential role of cyclin F mRNA expression in the survival of skin melanoma patients: Comprehensive analysis of the pathways altered due to cyclin F upregulation

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Received December 20, 2017; Accepted May 3, 2018

DOI: 10.3892/or.2018.6435

Abstract. Cyclin F is a part of the Skp, Cullin, F-box containing ligase complex. The activity of cyclin F includes cell cycle control, centrosome duplication and response to DNA damage. The cyclin F expression pattern is very similar to cyclin A, but cyclin F is an orphan cyclin without its cyclin-dependent kinase partner. There is little evidence concerning the role of cyclin F in cancer. In the present study, for the first time, we present analysis from The Cancer Genome Atlas (TCGA) data in the context of expression of cyclin F mRNA in melanoma patients. Our original *in silico* analysis, not published elsewhere before, revealed that high expression of cyclin F in melanoma patients is associated with worse overall survival. Cyclin F and ribonucleotide reductase family member 2 (RRM2) compose a functional axis responsible for nucleotide metabolism. Impairment in this pathway may contribute to increased DNA damage repair and drug resistance. Additionally, we analyzed the expression of RRM2 mRNA and discovered that high expression of RRM2 is associated with worse overall survival. To shed more light on cyclin F overexpression in melanoma, we analyzed all protein data available in the TCGA melanoma dataset. It was found that in patients with upregulated cyclin F mRNA, we noted increased activity of pathways related to cell cycle and DNA damage repair. These data will support further *in vitro* and *in vivo* studies on the involvement of cyclin F in skin cutaneous melanoma.

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Key words: cyclin F, CCNF, ribonucleotide reductase, melanoma, skin cancer

Introduction

Although melanoma comprises 5% of all skin-related tumors, it is responsible for 75% of the deaths caused by this type of cancer. Although significant progress has been made in the last decade and the number of cases has significantly decreased, the overall mortality rate has remained steady. New treatment strategies based on BRAF inhibitors or CTLA-4 blocking antibodies have provided only slight benefit to patients with stage IV melanoma and melanoma metastases. This moderate success provides the rationale to continue research on expanding therapies focusing on cancer biology and targeting molecular pathways crucial for proliferation, metastasis and respond to treatment (1-3).

DNA synthesis and repair require coordinated deoxyribonucleoside triphosphate (dNTP) supply as basic building blocks. Impaired balance of the dNTP pool affects S phase duration time, DNA synthesis fidelity, as well as the ability and effectiveness of DNA repair. Loss of control over these processes can also trigger genome instability and may initiate cancerogenesis. The increased demand for deoxyribonucleotides is serviced by upregulation of ribonucleotide reductase (RNR), which reduces the 2' carbon of a ribonucleoside diphosphate and has been considered as the rate-limiting step in dNTP production. RNR as a heterodimeric protein consists of three subunits – one ribonucleotide reductase family member 1 (RRM1) and two molecules of RRM2. While RRM1 expression is constant throughout the cell cycle, the expression of RRM2 fluctuates and peaks at S phase, when the need for nucleotide synthesis is the highest. The degradation of RRM2 occurs in late G2 phase of the cell cycle in the nucleus and is controlled by Skp, Cullin, F-box containing $(SCF)^{cyclin\ F}$ ubiquitin ligase complex. The SCF complex is composed of three proteins: Skp1 and Cul1, which provide a scaffold, and F-box protein, which is responsible for target recognition (4).

Cyclin F, like other cyclins, has both cyclin and F-box domains, but it does not bind or activate any known cyclin-dependent kinase (CDK). The expression profile of cyclin F is similar to cyclin A and fluctuates throughout the cell cycle. At the protein level, cyclin F appears in the S phase, peaks before M phase, and then its expression decreases dramatically. It is clearly visible that changes in the expression of cyclin F

negatively correlates with the RRM2 level, which may suggest their cooperation in the axis, important for genome stability and DNA repair (5). As it has been suggested, overexpression of RRM2 is associated with poorer patient prognosis in melanoma and many other cancers. Furthermore, cells with high content of RRM2 are characterized by much more effective DNA repair systems which impair the effectiveness of therapy (6-9).

The aim of our *in silico* analysis was to take the first step in the elucidation of the precise mechanism of the cyclin F (CCNF)-RRM2 axis in skin melanoma. The study aims to accelerate the development and to inspire other scientific teams to conduct similar research in the field.

In the present study, using the data available in the cBioPortal database, we showed for first time that high expression of cyclin F mRNA is associated with poorer prognosis in patients with skin cutaneous melanoma. Additionally, we present an overview of the molecular pathways involved in the cell cycle, cell death and DNA repair which are activated differentially in patients who exhibit high and low expression of cyclin F and RRM2.

Materials and methods

Analysis of publicly available data. To assess the expression profile of cyclin F and RMM2 mRNA, we obtained data from The Cancer Genome Atlas via www.cBioPortal.org (10). Patients were divided into groups: with CCNF or RRM2 mRNA upregulated expression (z-score >0) and with downregulated mRNA expression (z-score ≤0) and then, for each mRNA, we conducted overall survival and disease-free survival analysis. The same source was used for protein level comparison in patients with upregulated and downregulated cyclin F and RRM2 mRNA. In turn, we analyzed obtained information and used Reactome (http://reactome.org) and ToppGene Suite (http://toppgene.cchmc.org) to organize data into biological processes and functional molecular pathways.

Statistical analysis. In the life span study of the melanoma patients, the data were analyzed with Kaplan-Meier survival analysis with included log-rank test for trend tests. Comparisons between groups expressing different levels of mRNA or proteins were conducted using Mann-Whitney U-test. All statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

The TCGA data were used to characterize the prognostic value of cyclin F and RRM2 mRNA in melanoma. The results showed that increased expression of cyclin F mRNA is associated with worse outcome in melanoma patients (Fig. 1; Tables I and II). Median survival in patients with upregulated cyclin F was significantly lower (112.48 vs. 55.55 months; P<0.0001). No significance in disease-free survival (DFS) was found. Furthermore, expression of RRM2 mRNA had a significant influence on median survival (102.04 vs. 61.47; P=0.034), but no effect on DSF was noted (Fig. 2; Tables I and II). Cyclin F significantly altered the expression of different cellular proteins. The expression of proteins negatively and positively correlated with CCNF mRNA are listed in Tables III and V. Representative plots are shown in Figs. 3 and 4. Analogous data for RRM2 mRNA expression are shown in Tables VII and IX, and representative plots are presented in Figs. 5 and 6.

The analysis using Reactome showed that upregulation of cyclin F resulted in downregulation of pathways responsible for signal transduction and activation of cell cycle-related and DNA repair (Fig. 7). High expression of RRM2 mRNA also resulted in downregulation of cell signaling pathways. Activation of the cell cycle and DNA pathways was also visible but less univocal (Fig. 8). Upregulation of cyclin F coincides with altered expression of factors that were associated with worse patient outcome. Furthermore, patients with worse outcome had increased levels of proliferative proteins, such as cyclin E, cyclin B, PCNA, pro-survival factors such as p27 or FOXM1 and connected with AKT pathway activation (INPP4B). The list of biological processes altered by cyclin F dysregulation are presented in Tables IV and VI. Furthermore, data presenting biological processes influenced by changes in RRM2 expression are presented in Tables VIII and X.

Discussion

There is only limited data describing cyclin F and its possible role in human cancer. D'Angiolella *et al* characterized the functional axis which is responsible for DNA repair following genotoxic stress (5). It is possible that interaction between cyclin F and RRM2 is significantly responsible for treatment response, thus detailed recognition of its nature may be useful for cancer clinical outcome prediction. Nuclear accumulation of RRM2, which allows efficient DNA repair, is preceded by downregulation of cyclin F. As it has been shown by D'Angiolella *et al* the insertion of wild-type cyclin F into hTERT RPE-1 cells prevents transposition of RRM2 from the cytoplasm to the nucleus (5). It has also been shown that overexpression of RRM2 may affect the proliferation of melanoma cells, their response to treatment *in vivo*, and is associated with worse overall survival in melanoma patients bearing mutations in the BRAF oncogene (8,11,12). Based on these data, we hypothesized that low expression of cyclin F in melanoma patients can be related to a poorer prognosis. This hypothesis was strengthened by the fact that the relationship between low cyclin F expression and poorer prognosis was demonstrated by Fu *et al* in patients with hepatocellular carcinoma. They showed that downregulation of cyclin F in hepatocellular carcinoma tissue samples was related to larger tumor size and poor tumor differentiation (13). Interestingly our analysis revealed that high expression of cyclin F mRNA is associated with poorer prognosis in skin cutaneous melanoma. Much as the result differs from what was expected, it is not surprising as overexpression of cyclin proteins is more common in cancer rather than their downregulation. Sun *et al* showed that overexpression of cyclin B1 is associated with poorer prognosis and reduced overall survival in breast cancer (14). Li *et al* revealed an association between high expression of cyclin B1 and claudin-1 with worse outcome in patients with hypopharyngeal squamous cell carcinoma (15). On the other hand, high cyclin B1 expression was found to reduce lymph node metastasis and distant metastasis stage, and was also associated with higher survival rates in colorectal cancer (16). High

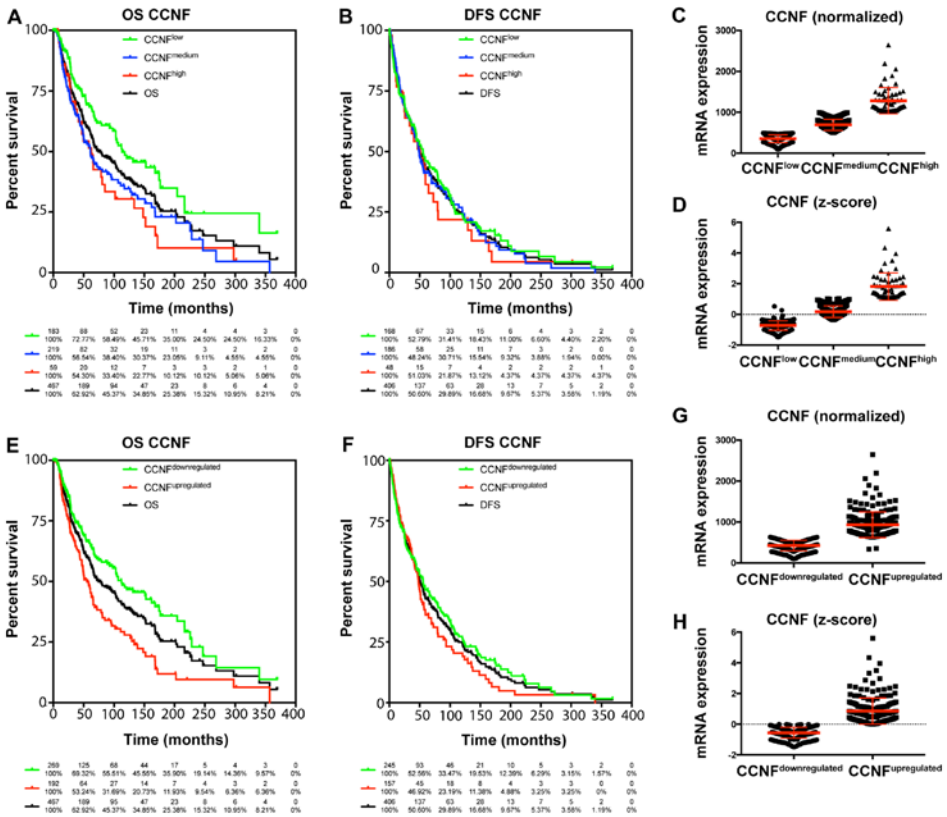

Figure 1. (A, B, E and F) High expression of CCNF mRNA is associated with poorer prognosis in melanoma patients. Patients with melanoma were analyzed by Kaplan-Meier survival estimation (log-rank test). (C, D, G and H) Representative plots of patients with differential expression of CCNF mRNA: normalized (C and G) and z-score (D and H). OS, overall survival; DFS, disease-free survival; CCNF, cyclin F.

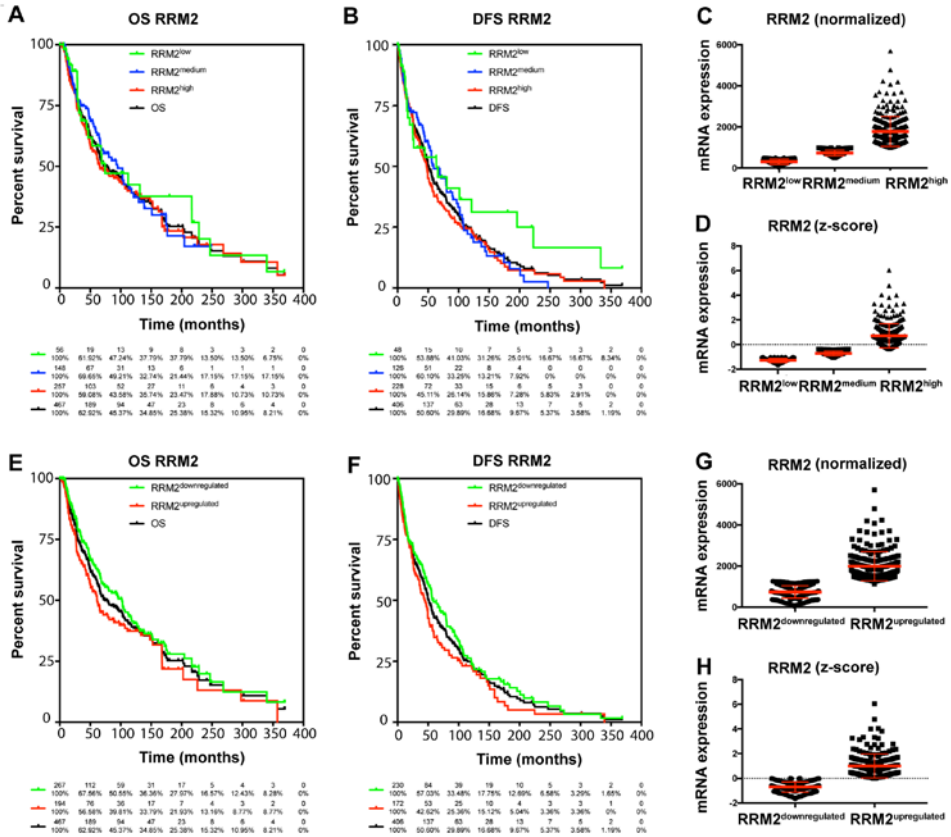

Figure 2. (A, B, E and F) High expression of RRM2 is associated with less favorable outcome in melanoma patients. Patients with melanoma were analyzed by Kaplan-Meier survival estimation (log-rank test). (C, D, G and H) Representative plots of patients with differential expression of RRM2: normalized (C and G) and z-score (D and H). OS, overall survival; DFS, disease-free survival; RRM2, ribonucleotide reductase family member 2.

Table I. Association of CCNF and RRM2 mRNA expression on the survival of melanoma patients.

Factor	Median survival (months)	Disease-free median survival (months)	Overall survival (%)			Disease-free survival (%)		
			5 years	10 years	15 years	5 years	10 years	15 years
Total	74.67	51.08	58.79	39.20	25.38	42.85	24.93	12.09
CCNF expression (normalized)								
$CCNF^{low}$	113.44	55.49	68.40	48.55	35.00	46.05	24.17	15.40
$CCNF^{medium}$	61.10	48.59	52.24	34.64	23.05	41.18	26.87	10.88
$CCNF^{high}$	62.75	51.08	51.44	30.36	10.12	36.45	21.87	4.37
CCNF expression (z-score)								
$CCNF^{downregulated}$	112.48	55.85	65.83	47.57	35.90	46.50	27.27	15.15
$CCNF^{upregulated}$	55.55	48.00	48.06	27.93	11.93	36.14	20.46	6.50
RRM2 expression (normalized)								
$RRM2^{low}$	74.67	63.40	58.48	42.51	37.79	53.88	36.47	31.26
$RRM2^{medium}$	94.91	58.97	64.07	39.11	21.44	49.07	22.17	10.57
$RRM2^{high}$	65.83	47.60	55.55	39.11	23.47	37.74	24.46	8.74
RRM2 expression (z-score)								
$RRM2^{downregulated}$	102.04	58.97	63.15	41.44	27.97	49.34	26.30	15.51
$RRM2^{upregulated}$	61.47	44.15	52.73	37.40	21.93	34.77	23.16	6.72

CCNF, cyclin F; RRM2, ribonucleotide reductase family member 2.

Table II. Changes in overall survival and disease-free survival as associated with CCNF and RRM2 mRNA expression in melanoma patients.

Factor	Overall survival				Disease-free survival			
	HR	95% CI	P-value	Significance	HR	95% CI	P-value	Significance
CCNF expression (normalized)								
$CCNF^{low}$ vs. total	0.73	0.57-0.93	0.0119	*	0.96	0.77-1.19	0.6915	NS
$CCNF^{medium}$ vs. total	1.21	0.96-1.54	0.1070	NS	1.01	0.81-1.27	0.9072	NS
$CCNF^{high}$ vs. total	1.33	0.90-1.97	0.1576	NS	1.11	0.75-1.62	0.6087	NS
$CCNF^{low}$ vs. $CCNF^{medium}$	0.60	0.45-0.80	0.0005	***	0.95	0.73-1.27	0.6733	NS
$CCNF^{low}$ vs. $CCNF^{high}$	0.48	0.30-0.77	0.0022	**	0.86	0.57-1.30	0.4748	NS
$CCNF^{medium}$ vs. $CCNF^{high}$	0.94	0.64-1.39	0.7717	NS	0.92	0.61-1.37	0.6784	NS
CCNF expression (z-score)								
$CCNF^{downregulated}$ vs. total	0.79	0.63-0.98	0.0317	*	0.94	0.78-1.15	0.5671	NS
$CCNF^{upregulated}$ vs. total	1.42	1.11-1.82	0.0053	**	1.11	0.87-1.40	0.3980	NS
$CCNF^{downregulated}$ vs. $CCNF^{upregulated}$	0.54	0.43-0.75	<0.0001	****	0.85	0.66-1.10	0.2211	NS
RRM2 expression (normalized)								
$RRM2^{low}$ vs. total	0.87	0.59-1.30	0.5052	NS	0.77	0.53-1.12	0.1693	NS
$RRM2^{medium}$ vs. total	0.93	0.71-1.22	0.5970	NS	0.96	0.75-1.24	0.7756	NS
$RRM2^{high}$ vs. total	0.95	0.76-1.17	0.6165	NS	1.08	0.88-1.32	0.4596	NS
$RRM2^{low}$ vs. $RRM2^{medium}$	1.10	0.68-1.76	0.7059	NS	1.31	0.85-2.03	0.2260	NS
$RRM2^{low}$ vs. $RRM2^{high}$	0.84	0.56-1.27	0.4156	NS	0.73	0.50-1.08	0.1134	NS
$RRM2^{medium}$ vs. $RRM2^{high}$	0.88	0.66-1.18	0.3845	NS	0.90	0.69-1.17	0.4161	NS
RRM2 expression (z-score)								
$RRM2^{downregulated}$ vs. total	0.88	0.71-1.09	0.2507	NS	1.11	0.91-1.36	0.3133	NS
$RRM2^{upregulated}$ vs. total	1.17	0.92-1.50	0.1960	NS	1.15	0.92-1.44	0.2233	NS
$RRM2^{downregulated}$ vs. $RRM2^{upregulated}$	0.75	0.57-0.98	0.0344	*	0.78	0.61-1.00	0.0529	NS

HR, hazard ratio; CI, confidence interval; CCNF, cyclin F; RRM2, ribonucleotide reductase family member 2. ****, extremely significant (P<0.0001); ***, extremely significant (P=0.0001 to 0.001); **, very significant (P=0.001 to 0.01); *, significant (P=0.01 to 0.05); NS, not significant (P≥0.05).

Table III. Expression of proteins which are negatively correlated with CCNF.

		$CCNF^{downregulated}$	$CCNF^{upregulated}$		
		RPPA (z-score)			
Protein	Gene	upregulated	downregulated	P-value	Significance
A-Raf_pS299	ARAF	0.0567	-0.0105	0.0279	*
Annexin_VII	ANXA7	0.0085	-0.0491	0.0055	**
Annexin-1	ANXA1	0.2359	-0.0402	0.0006	***
AR	AR	0.0662	-0.0072	0.0380	*
Axl	AXL	0.1741	-0.0276	0.0283	*
Bak	BAK1	0.0059	-0.0199	0.5374	NS
Bcl-2	BCL2	0.0461	-0.1069	0.0190	*
Bcl-xL	BCL2L1	0.0578	-0.0127	0.0609	NS
Bim	BCL2L11	0.0081	-0.1046	0.0200	*
Caveolin-1	CAV1	0.2809	-0.0344	0.0013	**
CD31	PECAM1	0.0548	-0.0108	0.0260	*
CD49b	ITGA2	0.1129	-0.0100	<0.0001	****
Chk1_pS345	CHEK1	0.0011	-0.0009	0.6241	NS
DJ-1	PARK7	0.0503	-0.0112	0.0743	NS
EGFR_pY1068	EGFR	0.0817	-0.0107	0.0015	**
ER-α	ESR1	0.0900	-0.0314	0.0002	***
FOXO3a	FOXO3	0.0724	-0.0102	<0.0001	****
GATA3	GATA3	0.0186	-0.0356	0.0287	*
GATA6	GATA6	0.0949	-0.0295	0.0132	*
HER2	ERBB2	0.0678	-0.0827	0.0036	**
HER3	ERBB3	0.0023	-0.0620	0.2038	NS
HER3_pY1289	ERBB3	0.0086	-0.0137	0.1953	NS
INPP4B	INPP4B	0.0761	-0.0258	0.0008	***
JAB1	COPS5	0.0558	-0.1180	<0.0001	****
JNK2	MAPK9	0.0404	-0.0589	0.0083	**
Myosin-IIa	MYH9	0.0003	-0.0030	0.9509	NS
p27	CDKN1B	0.0582	-0.1027	<0.0001	****
p38_pT180_Y182	MAPK14	0.0115	-0.0346	0.3252	NS
p53	TP53	0.0557	-0.0223	0.0021	**
PARP_cleaved	PARP1	0.0227	-0.0241	0.0773	NS
PDCD4	PDCD4	0.0854	-0.1255	0.0025	**
PEA15	PEA15	0.0238	-0.0052	0.4346	NS
PI3K-p110-α	PIK3CA	0.0097	-0.0625	0.0315	*
PKC-α	PRKCA	0.1358	-0.2574	<0.0001	****
PKC-α_pS657	PRKCA	0.1951	-0.1638	<0.0001	****
PKC-δ_pS664	PRKCD	0.0194	-0.0539	0.1518	NS
PRDX1	PRDX1	0.0266	-0.0393	0.2556	NS
Rab25	RAB25	0.0432	-0.0767	0.0011	**
Rad50	RAD50	0.0579	-0.0170	0.1381	NS
Shc_pY317	SHC1	0.0064	-0.0834	0.0026	**
Src_pY416	SRC	0.0296	-0.0103	0.4421	NS
VEGFR2	KDR	0.0142	-0.0164	0.3048	NS

CCNF, cyclin F; RPPA, reverse-phase protein array. ****, extremely significant (P<0.0001); ***, extremely significant (P=0.0001 to 0.001); **, very significant (P=0.001 to 0.01); *, significant (P=0.01 to 0.05); NS, not significant (P≥0.05).

Table IV. Biological process and pathway analysis of genes whose products are negatively correlated with CCNF expression.

Factor	P-value	Number of genes	Gene list
Biological process			
Regulation of apoptotic process	1.26E-12	19	GATA3, GATA6, CDKN1B, FOXO3, PRKCA, ERBB2, BCL2, CAV1, MAPK9, BCL2L11, EGFR, PIK3CA, ANXA1, AXL, AR, ARAF, PDCD4, ESR1, TP53
Regulation of intracellular signal transduction	7.99E-12	19	SHC1, GATA3, PRKCA, ERBB2, BCL2, CAV1, MAPK9, BCL2L11, EGFR, PIK3CA, COPS5, AXL, AR, ARAF, PDCD4, ESR1, INPP4B, TP53, PECAM1
Apoptotic process	2.63E-11	19	GATA3, GATA6, CDKN1B, FOXO3, PRKCA, ERBB2, BCL2, CAV1, MAPK9, BCL2L11, EGFR, PIK3CA, ANXA1, AXL,AR, ARAF, PDCD4, ESR1, TP53
Negative regulation of apoptotic process	3.01E-11	15	GATA3, GATA6, CDKN1B, PRKCA, ERBB2, BCL2,CAV1, EGFR, PIK3CA,ANXA1, AXL, AR, ARAF, PDCD4, TP53
Positive regulation of cellular protein metabolic process	1.08E-10	17	SHC1, GATA3, CDKN1B, PRKCA, ERBB2, BCL2, ITGA2, CAV1, MAPK9, BCL2L11, EGFR, PIK3CA, AR, ARAF, ESR1, TP53, PECAM1
Regulation of protein modification process	1.30E-10	18	SHC1, GATA3, CDKN1B, PRKCA, ERBB2, BCL2, ITGA2, CAV1, MAPK9, EGFR, PIK3CA, COPS5, AR, ARAF, PDCD4, ESR1, TP53, PECAM1
Positive regulation of signaling	3.37E-10	17	SHC1, GATA3, GATA6, PRKCA, ERBB2, BCL2, ITGA2, CAV1, MAPK9, BCL2L11, EGFR, AXL, AR, ARAF, ESR1, TP53, PECAM1
Positive regulation of cell communication	3.61E-10	17	SHC1, GATA3, GATA6, PRKCA, ERBB2, BCL2, ITGA2, CAV1, MAPK9, BCL2L11, EGFR, AXL, AR, ARAF, ESR1, TP53, PECAM1
Regulation of phosphorylation	7.58E-10	16	SHC1, CDKN1B, PRKCA, ERBB2, BCL2, CAV1, MAPK9, EGFR, PIK3CA, COPS5, AR, ARAF, PDCD4, ESR1, TP53, PECAM1
Positive regulation of phosphorylation	7.76E-10	14	SHC1, CDKN1B, PRKCA, ERBB2, BCL2, CAV1, MAPK9, EGFR, PIK3CA, AR, ARAF, ESR1, TP53, PECAM1
Regulation of cell proliferation	2.67E-09	16	SHC1, GATA3, GATA6, CDKN1B, FOXO3, PRKCA, ERBB2, BCL2, RAB25, ITGA2, CAV1, EGFR, ANXA1, AR, ESR1, TP53
Positive regulation of cell proliferation	4.30E-09	13	SHC1, GATA6, CDKN1B, PRKCA, ERBB2, BCL2, RAB25, ITGA2, CAV1, EGFR, ANXA1, AR, ESR1
Cell adhesion	1.11E-07	14	SHC1, GATA3, PRKCA, ERBB2, BCL2, ITGA2, CAV1, BCL2L11, EGFR, PIK3CA, ANXA1, AXL, TP53, PECAM1
Positive regulation of apoptotic process	3.01E-07	10	GATA6, CDKN1B, FOXO3, BCL2, CAV1, MAPK9, BCL2L11, ANXA1, PDCD4, TP53
Pathway			
EGFR tyrosine kinase inhibitor resistance	2.50E-13	10	SHC1, FOXO3, PRKCA, ERBB2, BCL2, BCL2L11, EGFR, PIK3CA, AXL, ARAF
Endocrine resistance	9.60E-13	10	SHC1, CDKN1B, ERBB2, BCL2, MAPK9, EGFR,PIK3CA, ARAF, ESR1, TP53
Proteoglycans in cancer	1.01E-09	10	PRKCA, ERBB2, ITGA2, CAV1, EGFR, PIK3CA, ARAF, PDCD4, ESR1, TP53
ErbB signaling pathway	1.01E-09	8	SHC1,CDKN1B,PRKCA,ERBB2,MAPK9,EGFR,PIK3CA, ARAF
Focal adhesion	1.57E-08	9	SHC1, PRKCA, ERBB2, BCL2, ITGA2, CAV1, MAPK9, EGFR, PIK3CA

Table IV. Continued.

Factor	P-value	Number of genes	Gene list
Pathways in cancer	1.57E-08	11	CDKN1B, PRKCA, ERBB2, BCL2, ITGA2, MAPK9, EGFR, PIK3CA, AR, ARAF, TP53
MicroRNAs in cancer	2.01E-08	10	SHC1, CDKN1B, PRKCA, ERBB2, BCL2, BCL2L11, EGFR, PIK3CA, PDCD4, TP53
FoxO signaling pathway	4.15E-07	7	CDKN1B, FOXO3, MAPK9, BCL2L11, EGFR, PIK3CA, ARAF
Signaling by SCF-KIT	7.61E-07	9	SHC1, CDKN1B, FOXO3, PRKCA, ERBB2, EGFR, PIK3CA, ARAF, TP53
PI3K-Akt signaling pathway	7.61E-07	9	CDKN1B, FOXO3, PRKCA, BCL2, ITGA2, BCL2L11, EGFR, PIK3CA, TP53
Signaling by NGF	9.31E-07	10	SHC1, CDKN1B, FOXO3, PRKCA, ERBB2, BCL2L11, EGFR, PIK3CA, ARAF, TP53
HIF-1 signaling pathway	1.43E-06	6	CDKN1B, PRKCA, ERBB2, BCL2, EGFR, PIK3CA
Apoptosis signaling pathway	1.43E-06	6	PRKCA, BCL2, MAPK9, BCL2L11, PIK3CA, TP53
Signaling by ERBB2	1.54E-06	5	SHC1, PRKCA, ERBB2, EGFR, PIK3CA

CCNF, cyclin F; SCF, Skp, Cullin, F-box containing. P-values were corrected for multiple comparisons using the false discovery rate (FDR) (Benjamini and Hochberg).

expression of cyclin D1 is a poor prognostic factor in gastric, oropharyngeal and breast cancer (17-19). Additionally, the overexpression of cyclin E correlates with worse outcome in patients with breast cancer, rectal cancer and gastrointestinal cancer (20-22). Some evidence has shown that low expression of cyclin F may be tumorigenic. It has been proposed that the downregulation of cyclin F promotes centrosomal and mitotic abnormalities associated with impaired degradation of CP110, an important centriolar protein (23). Moreover, cyclin F-mediated degradation of CDC-6 suppresses genome instability and prevents re-replication, limiting the number of cells with DNA content greater than 4N (24). Pan *et al* showed that different levels of cyclin F, cyclin D and RBL1 between 2D and 3D cultured cells may be associated with radioresistance of cells in 3-dimensional culture. They noted that A549 cells cultured in 3D exhibited lower levels of cyclin F and were less susceptible to G2/M cell cycle arrest after X-ray irradiation (25). However, the potential role of cyclin F as a tumor-promoting factor and the underlying mechanism remain elusive. The Oct4/NIPP1-CCNF/PP1 axis is responsible for maintenance of retinoblastoma protein 1 (Rb1) in the hyperphosphorylated state providing stem cell self-renewal and increased proliferation. Inactivation of Rb1 via CCNF/PP1 is also associated with enhanced ovarian cancer aggressiveness (26,27). In our pathway analysis, we observed a decrease in the cell signaling-related pathway activity and increase in the cell cycle-related pathways in patients with upregulated levels of cyclin F. A recent report showed that cyclin F is a bridge between AKT kinase and cell cycle machinery. Choudhury *et al* hypothesized a model where growth signaling initiates a positive loop where AKT phosphorylates and stabilizes cyclin F in the SCF complex. This stabilization inhibits degradation of cyclin F via APC/C (Cdh1) complex and promotes SCF-dependent degradation of Cdh1. Degradation of Cdh1 is essential for S phase entry and loss of cyclin F impairs cell cycle progression (28,29). Activation of the PI3K/AKT pathway is a common event in a variety of cancer diseases and it is believed to contribute to drug resistance. Although, we did not observe clear symptoms of PI3K/AKT activation, our analysis revealed downregulated INPP4B, tumor suppressor antagonizing PI3K/AKT pathway. Loss of INPP4B was found to increase AKT activation and drive higher proliferation rate and metastasis (30). It has been also reported that a decreased level of INPP4B is releted to higher proliferative, invasive and metastatic potential of melanocytic neoplasms (31). In contradiction to these reports is a study by Chi *et al* where upregulation of INPP4B in a melanoma subset was observed. Furthermore, INPP4B driven proliferation was Akt-independent and was mediated by serum- and glucocorticoid-regulated kinase 3 (SGK3). Additionally, they observed no significant differences between primary and metastatic melanoma suggesting the involvement of INPP4B in developing cancer from the early stages (32).

In the present study, the upregulation of cyclin F mRNA was found to coincide with the downregulation of p27 protein, important cell cycle regulator involved in G1 arrest. Akman *et al* found that patients with melanoma are characterized by lower p27 expression in comparison to patients with benign nevi and dysplastic nevi (33). Furthermore, Florenes *et al* reported that decreased expression of p27 is associated with increasing Breslow thickness and lower disease-free survival rates in primary nodular melanoma (34). Additionally, the low expression of p27 in melanocytic lesions may be responsible for its high proliferation rate (35). The lack

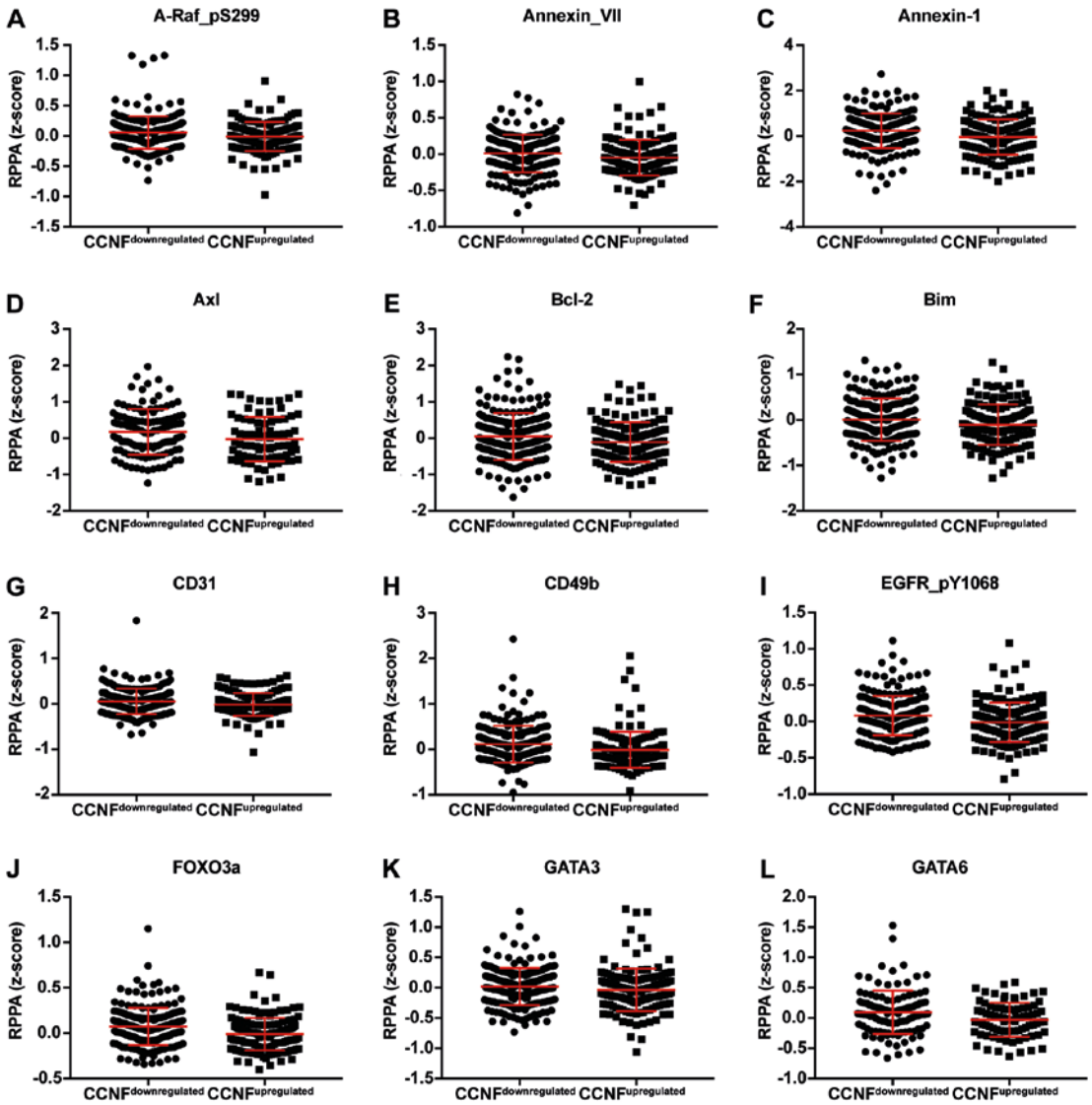

Figure 3. Dot plot representation of the protein levels by RPPA (z-score). (A-L) Proteins negatively correlated with CCNF mRNA. Horizontal bars represent lower quartile, median and higher quartile. CCNF, cyclin F; RPPA, reverse-phase protein array.

of proper control in regards to cell cycle events is typical for cancer cells. As was mentioned in the introduction, the overexpression of cyclins is very common in various malignancies. In our analysis, elevated levels of cyclin F mRNA were also associated with upregulation of cyclin E1 and B1 proteins. Elevated levels of cyclin E1 were observed in melanoma and enhanced expression of cyclin E was noted in both primary and metastatic melanomas. In contrast, its overexpression was not observed in non-malignant nevi (36). Bales *et al* reported that cyclin E is overexpressed in melanoma and present in the low-molecular form. Noteworthy, transfection of a primary cutaneous melanoma cell line with low tumorigenic and metastatic potential with low-molecular cyclin E forms resulted in the development of angiogenic tumors with prominent perineural invasion. Additionally, truncated forms of cyclin E triggered a dramatic increase in a number of metastasis events (37). In turn, cyclin B1 is involved in proliferation and metastatic potential of melanoma cells (38). Silencing of cyclin B exerts an antitumor effect on melanoma cells and lung metastases, both *in vitro* and *in vivo* (39).

Kruiswijk *et al* reported that patients with elevated levels of cyclin B1, Pin1 and FOXM1 display a worse outcome and exhibit increased mortality (40). FOXM1 is a pro-proliferative and pro-survival transcription factor participating in DNA repair. Moreover, these data are in agreement with our analysis, where a significant increase in FOXM1 protein in patients with upregulated cyclin F mRNA was noted. It suggests possible activation of cyclin F expression by FOXM1, but further research is needed to clarify this. Moreover, the upregulation of FOXM1 coincides with downregulation of FOXO3a. The abrogation of FOXO3a function was found to lead to increased tumor aggressiveness in melanoma and renal carcinoma (41,42). Another important observation made in this study is that 4E-BP1 (4E binding protein 1) was hyperphosphorylated in patients with upregulated cyclin expression. Phosphorylation of 4E-BP1 results in dissociation from translation factor eIF4E and allows cap-dependent translation. Phospho-4E-BP1 may also be useful as a marker of mTOR pathway activity and integrates signals obtained from PI3K/AKT and RAS/RAF/MEK/ERK pathways (43).

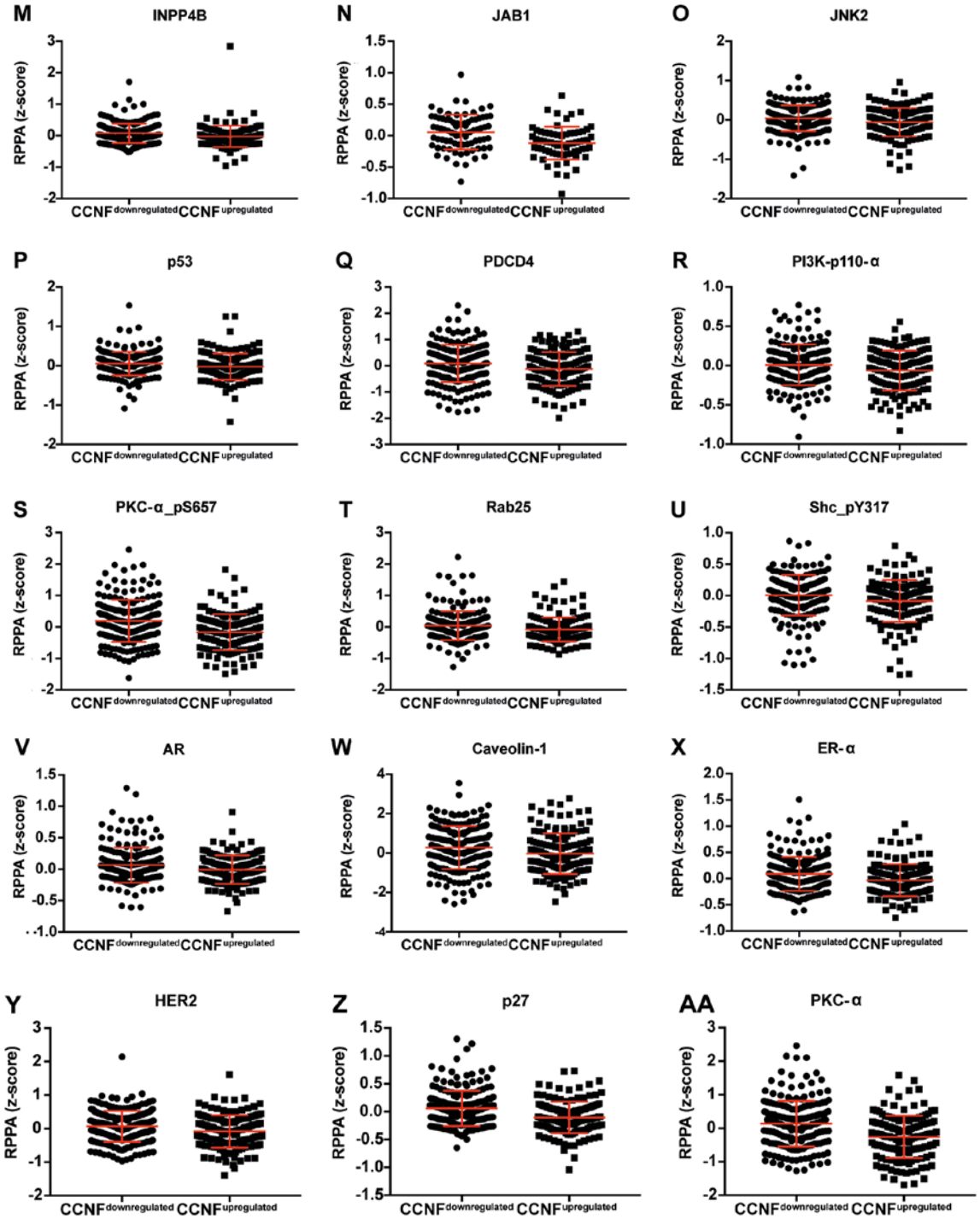

Figure 3. Continued. Dot plot representation of the protein levels by RPPA (z-score). (M-AA) Proteins negatively correlated with CCNF mRNA. Horizontal bars represent lower quartile, median and higher quartile. CCNF, cyclin F; RPPA, reverse-phase protein array.

Additionally, concomitant hyperphosphorylation of 4E-BP1 and activation of the PI3K/AKT pathway results in resistance to mTOR inhibitors. Moreover, in hypoxic conditions, 4E-BP1 initiates translation of proteins responsible for angiogenesis (VEGF-A), hypoxia response (HIF1α) and apoptosis resistance (Bcl-2) in advanced cancer (44,45). Increased levels of phosphorylated 4E-BP1 are also associated with poor overall survival and significant difference in post-recurrence survival (46). It is possible that cyclin F is a part of the specific cellular environment, promoting cell proliferation and survival.

The ability of cancer cells to efficiently repair DNA is a significant barrier to successful treatment. RRM2 is a part of the RNR and has been reported to be partially responsible for chemoresistance of cancer cells, including melanoma. However, our analysis did not reveal significant changes in overall survival or disease-free survival between patients with differential RRM2 mRNA expression. Aird *et al* showed that high RRM2 expression is correlated with worse outcome in melanoma patients (8). Silencing of RRM2 inhibited melanoma growth which suggests the involvement of RRM2 in melanoma progression. Silencing of RRM2 and treatment

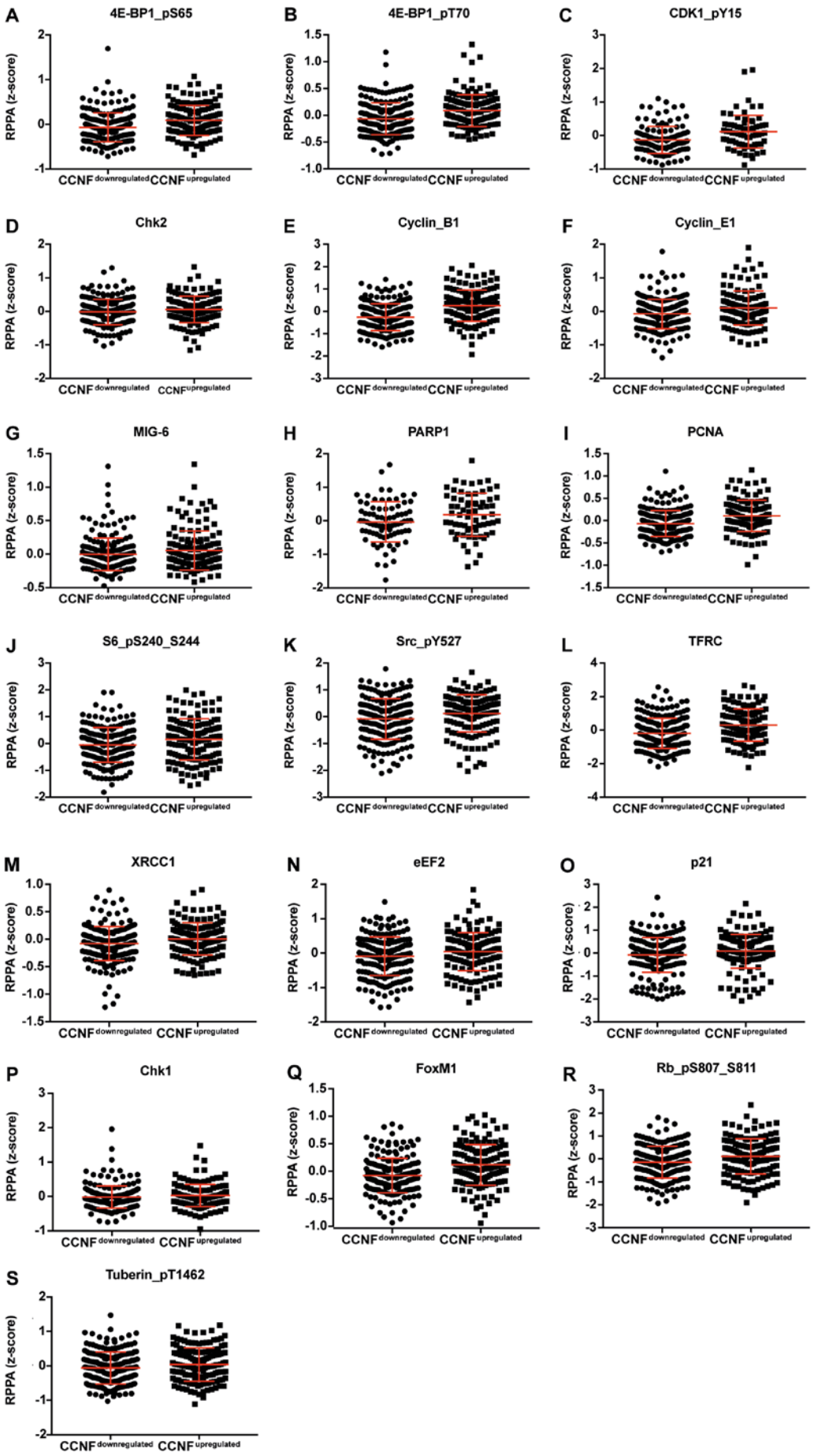

Figure 4. Dot plot representation of the protein levels by RPPA (z-score). (A-S) Proteins positively correlated with CCNF mRNA. Horizontal bars represent lower quartile, median and higher quartile. CCNF, cyclin F; RPPA, reverse-phase protein array.

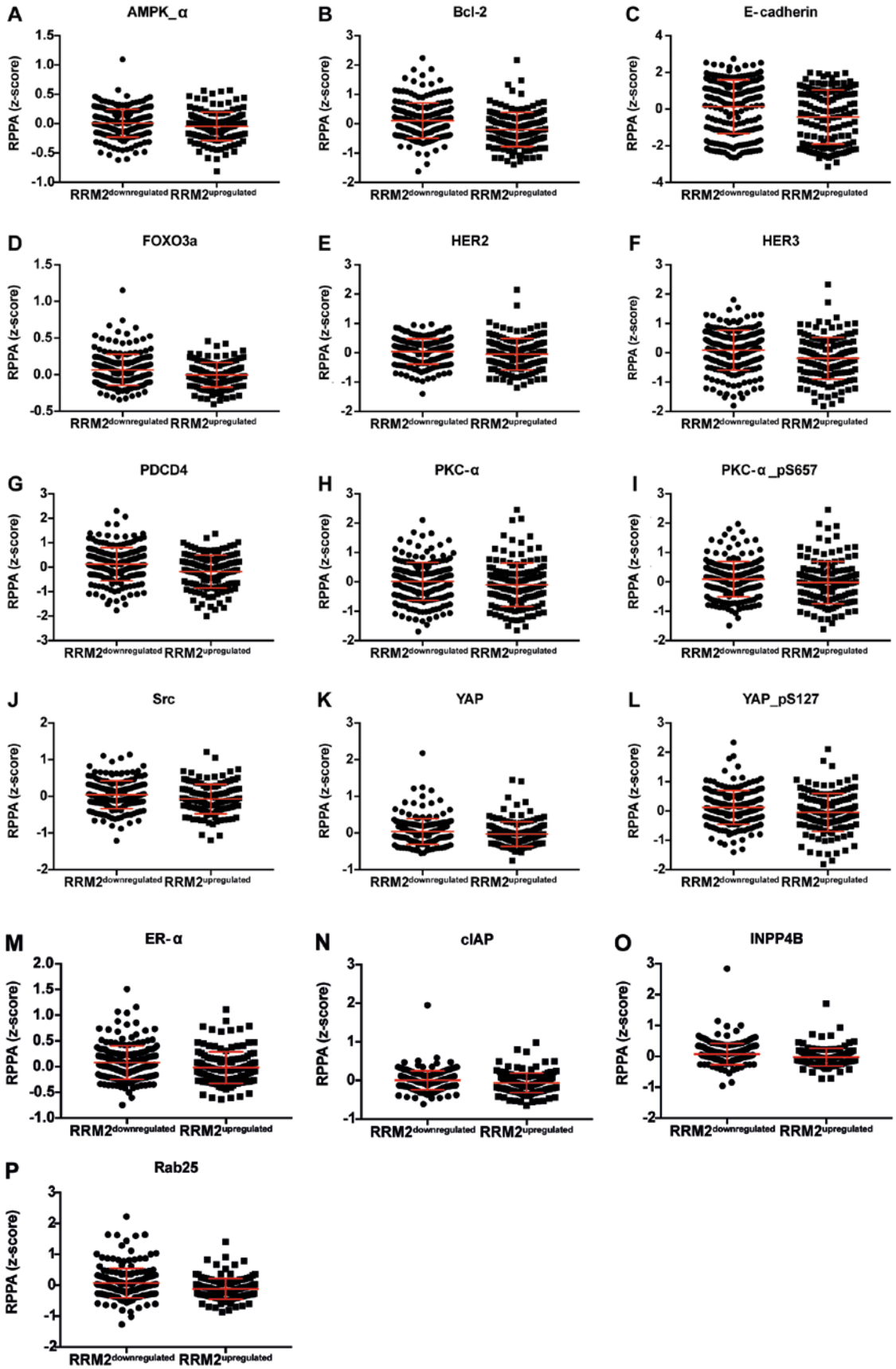

Figure 5. Dot plot representation of the protein levels by RPPA (z-score). (A-P) Proteins negatively correlated with RRM2 mRNA. Horizontal bars represent lower quartile, median and higher quartile. RRM2, ribonucleotide reductase family member 2; RPPA, reverse-phase protein array.

with mutant BRAF inhibitor PLX4720 simultaneously and synergistically inhibited melanoma growth (11). It is possible that the negative effect of RRM2 overexpression is limited to patients bearing $BRAF^{V600E}$ mutation, but we cannot confirm

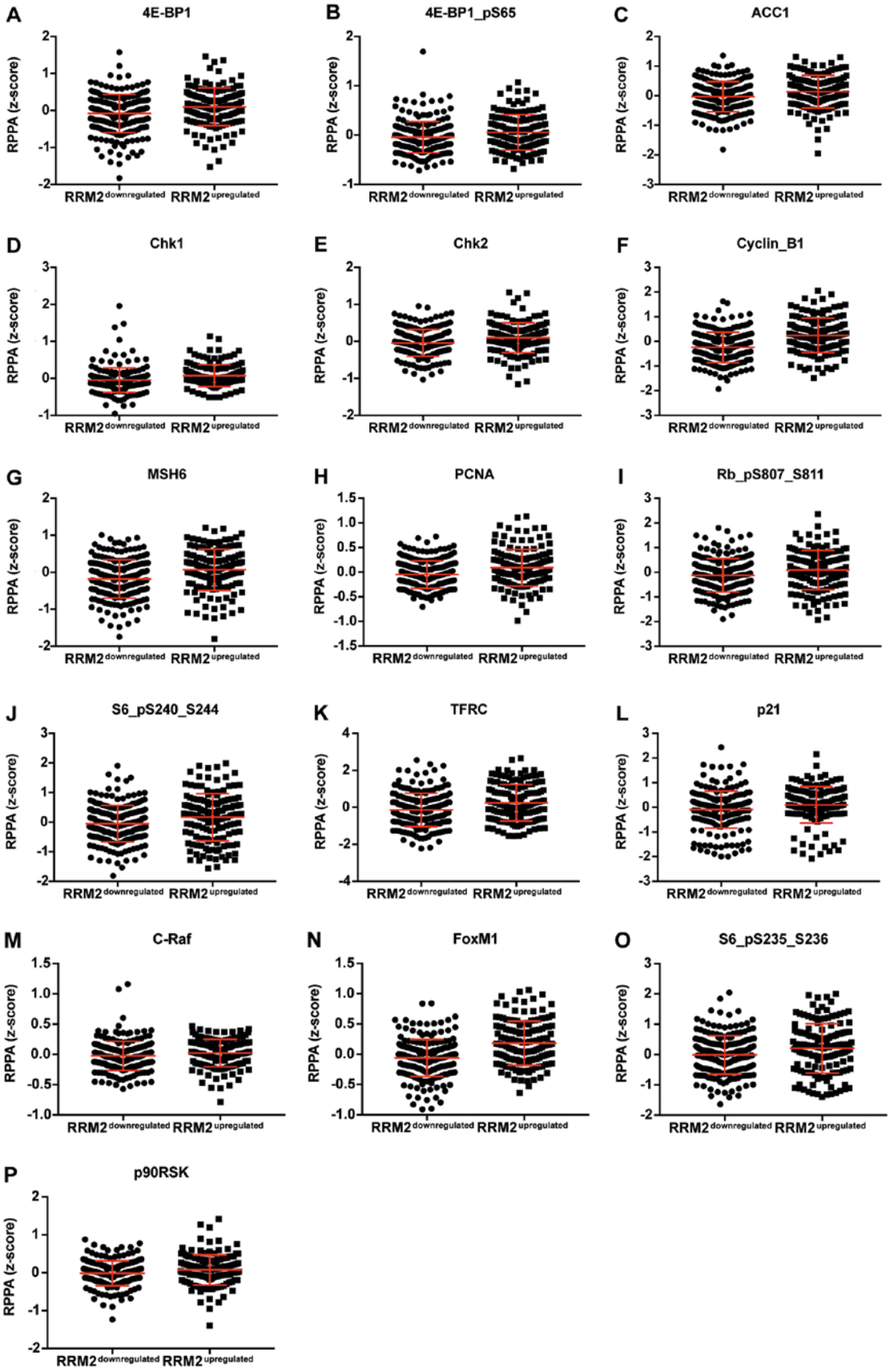

Figure 6. Dot plot representation of the protein levels by RPPA (z-score). (A-P) Proteins positively correlated with RRM2 mRNA. Horizontal bars represent lower quartile, median and higher quartile. RRM2, ribonucleotide reductase family member 2; RPPA, reverse-phase protein array.

this using TCGA data due to an insufficient number of patients with the BRAF mutation in the cohort.

Beyond controlling RRM2 levels, cyclin F is a limiting factor in histone H2.AX signalization. In the G2 phase

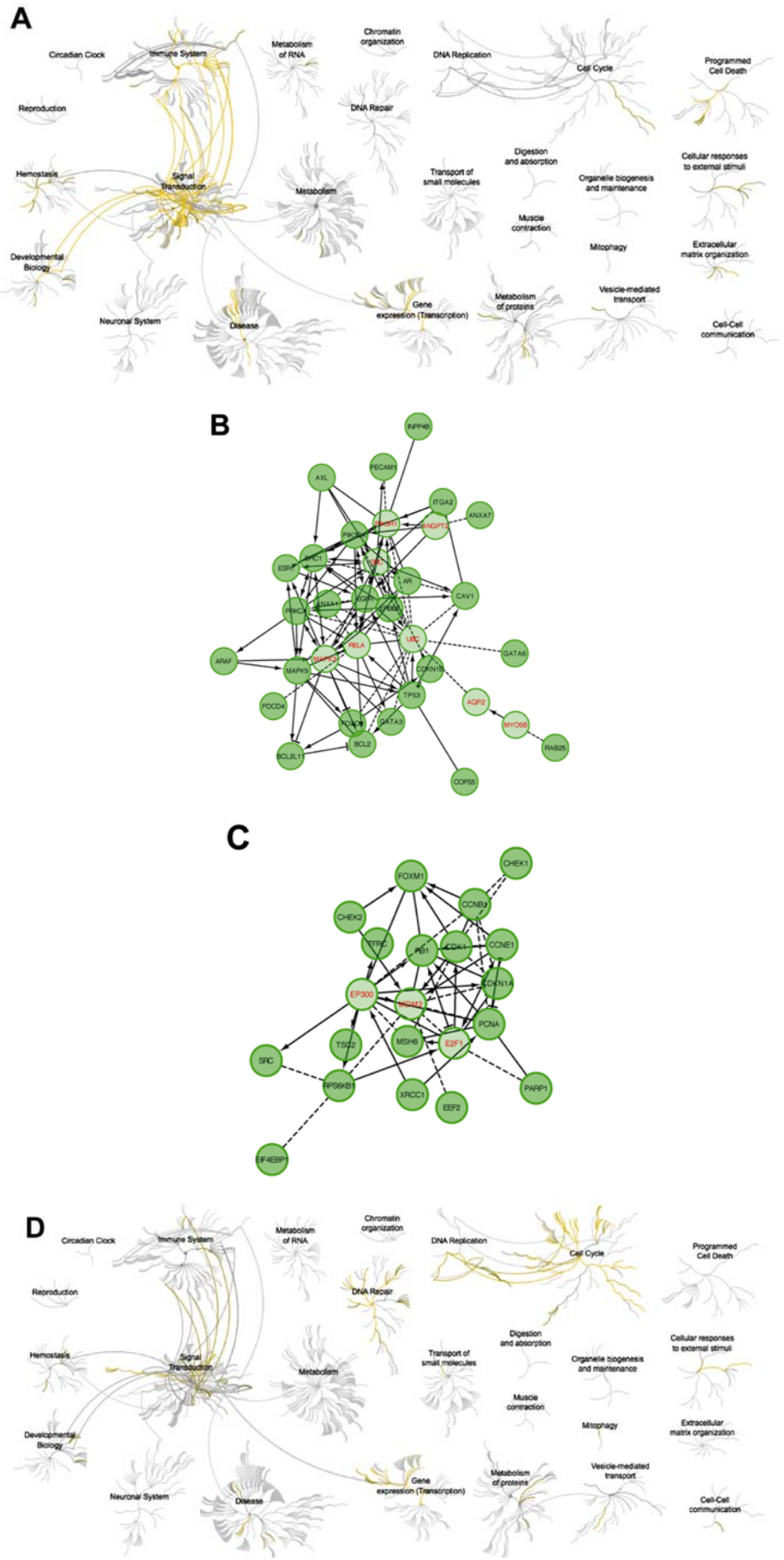

Figure 7. (A) Pathways negatively correlated with CCNF expression. (B) Relationships between proteins negatively correlated with CCNF expression involved in pathway analysis. (C) Pathways positively correlated with CCNF expression. (D) Relationships between proteins positively correlated with CCNF expression. CCNF, cyclin F.

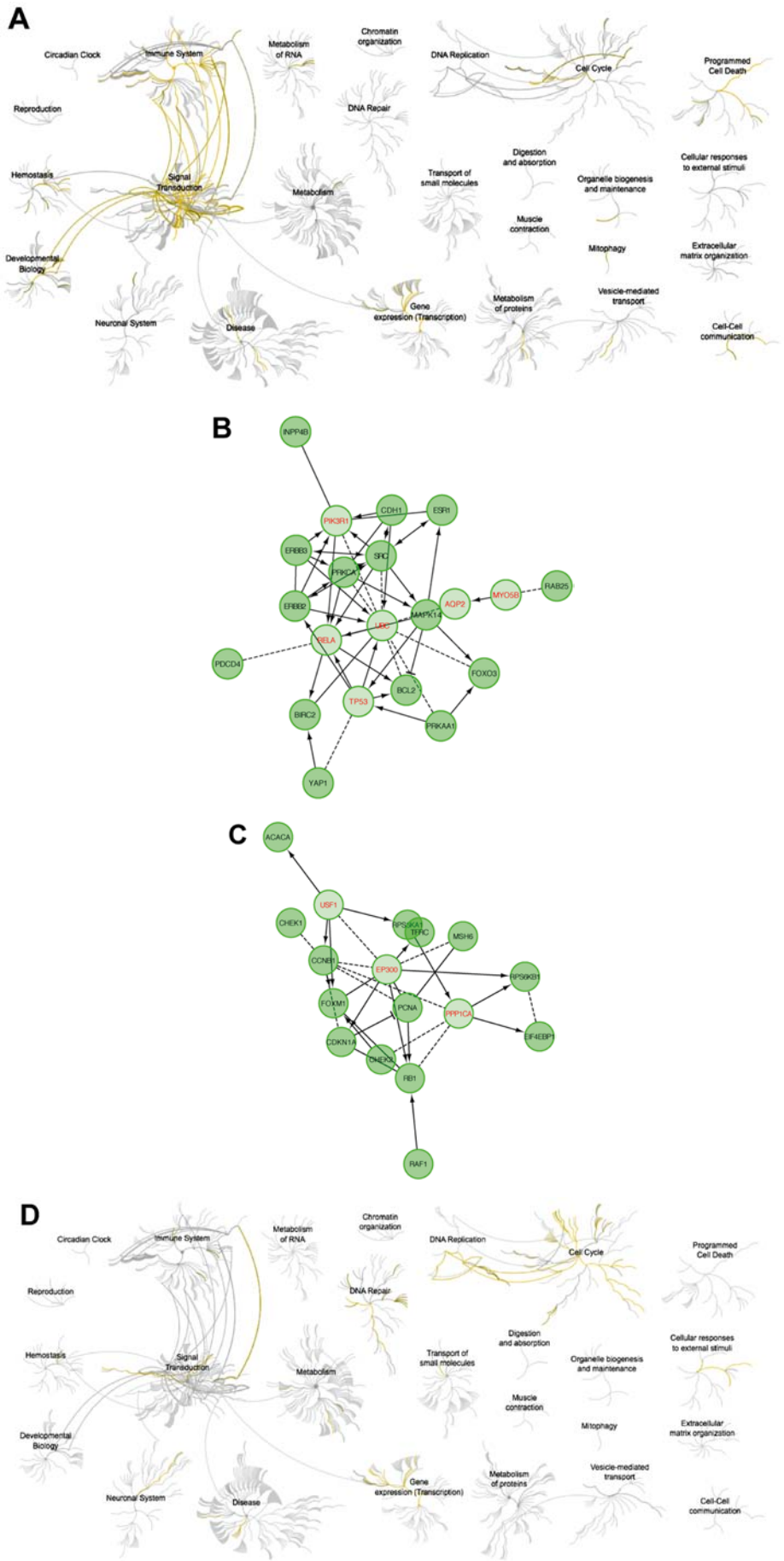

Figure 8. (A) Pathways negatively correlated with RRM2 expression. (B) Relationships between proteins negatively correlated with RRM2 expression involved in pathway analysis. (C) Pathways positively correlated with RRM2 expression. (D) Relationships between proteins positively correlated with RRM2 expression. RRM2, ribonucleotide reductase family member 2.

Table V. Expression of proteins which positively correlate with CCNF.

		$CCNF^{downregulated}$	$CCNF^{upregulated}$		
		RPPA (z-score)			
Protein	Gene	Downregulated	Upregulated	P-value	Significance
4E-BP1	EIF4EBP1	-0.0327	0.0285	0.3383	NS
4E-BP1_pS65	EIF4EBP1	-0.0677	0.0891	<0.0001	****
4E-BP1_pT70	EIF4EBP1	-0.0655	0.0860	<0.0001	****
ACC_pS79	ACACA	-0.0054	0.0362	0.3200	NS
C-Raf	RAF1	-0.0110	0.0031	0.3485	NS
CDK1_pY15	CDK1	-0.1318	0.1145	<0.0001	****
Chk1	CHEK1	-0.0156	0.0301	0.0333	*
Chk2	CHEK2	-0.0137	0.0578	0.0421	*
Cyclin_B1	CCNB1	-0.2619	0.2546	<0.0001	****
Cyclin_E1	CCNE1	-0.0766	0.1020	0.0009	***
eEF2	EEF2	-0.0851	0.0434	0.0449	*
FoxM1	FOXM1	-0.0423	0.1525	<0.0001	****
GAPDH	GAPDH	-0.0492	0.0336	0.5952	NS
MIG-6	ERRFI1	-0.0025	0.0536	0.1141	NS
MSH2	MSH2	-0.0296	0.0032	0.3485	NS
MSH6	MSH6	-0.1614	0.0359	0.0003	***
NF-kB-p65_pS536	NFKB1	-0.0494	0.0028	0.6642	NS
NF2	NF2	-0.0131	0.0148	0.7265	NS
p21	CDKN1A	-0.0769	0.0860	0.0186	*
p38_MAPK	MAPK14	-0.0104	0.0141	0.7464	NS
p62-LCK-ligand	SQSTM1	-0.0667	0.0042	0.1143	NS
PARP1	PARP1	-0.0340	0.1803	0.0425	*
PCNA	PCNA	-0.0654	0.1086	<0.0001	****
PRAS40_pT246	AKT1S1	-0.0248	0.0148	0.1293	NS
Rb_pS807_S811	RB1	-0.1385	0.1091	0.0014	**
S6_pS240_S244	RPS6KB1	-0.0474	0.1577	0.0086	**
SLC1A5	SLC1A5	-0.0542	0.0092	0.2129	NS
Src	SRC	-0.0137	0.0101	0.4254	NS
Src_pY527	SRC	-0.0814	0.1241	0.0022	**
TFRC	TFRC	-0.1894	0.3049	<0.0001	****
Tuberin_pT1462	TSC2	-0.0626	0.0410	0.0488	*
XRCC1	XRCC1	-0.0784	0.0078	0.0065	**

CCNF, cyclin F; RPPA, reverse-phase protein array. ****, extremely significant (P<0.0001); ***, extremely significant (P=0.0001 to 0.001); **, very significant (P=0.001 to 0.01); *, significant (P=0.01 to 0.05); NS, not significant (P≥0.05).

cyclin F mediates degradation of SLBP protein which promotes synthesis of H2AFX mRNA. Presence of SLBP in the G2 phase increases H2.AX levels and makes the cell more susceptible to apoptosis under genotoxic stress. It is another piece of evidence showing how cyclin F promotes cancer progression (47). Moreover, we observed an alteration in expression of other DNA-repair related proteins: XRCC1, PARP1, PCNA, and MSH6. All proteins were upregulated which is a hallmark of efficient DNA repair systems and a potential obstacle to successful treatment. However, the prognostic status of XRCC1 is ambiguous. Its overexpression is associated with less favorable prognosis in head and neck squamous carcinoma. Decreased levels of XRCC1 are responsible for acute side-effects after radiotherapy in breast cancer patients. Loss of XRCC1 confers a more aggressive phenotype in melanoma (48-50). It suggests an indirect effect of cyclin F overexpression on the DNA damage repair system. Additionally, PCNA in patients with upregulated cyclin F is very significantly increased, what confirms the higher proliferation potential of cells overexpressing cyclin F. These findings confirm a study by Wang *et al* in which treatment of cells with stimulatory polysaccharides from abalone, significantly increased the expression of cyclin B1, CDK1 and cyclin F (51).

Another interesting observation was increased expression of TFRC (transferrin receptor 1) gene in patients with high expression of cyclin F and RRM2 mRNA. It has been

Table VI. Biological process and pathway analysis of genes whose products are positively correlated with CCNF expression.

Factor	P-value	Number of genes	Gene list
Biological process			
Regulation of cell cycle	1.88E-10	13	CHEK2, FOXM1, CCNE1, CDKN1A, RB1, TSC2, RPS6KB1, CDK1, CHEK1, PCNA, EIF4EBP1, SRC, CCNB1
Cell cycle phase transition	1.94E-10	11	CHEK2, FOXM1, CCNE1, CDKN1A, RB1, RPS6KB1, CDK1, CHEK1, PCNA, EIF4EBP1, CCNB1
Cell cycle G1/S phase transition	2.22E-10	9	CHEK2, CCNE1, CDKN1A, RB1, RPS6KB1, CDK1, PCNA, EIF4EBP1, CCNB1
Positive regulation of cell cycle	1.82E-09	9	CHEK2, CDKN1A, RB1, RPS6KB1, CDK1, PCNA, EIF4EBP1, SRC, CCNB1
Regulation of DNA metabolic process	2.32E-09	9	CHEK2, FOXM1, CDKN1A, MSH6, PARP1, CDK1, CHEK1, PCNA, SRC
Cell cycle arrest	4.10E-09	8	CHEK2, FOXM1, CDKN1A, RB1, TSC2, CDK1, PCNA, CCNB1
Negative regulation of mitotic cell cycle phase transition	8.06E-09	7	CHEK2, CDKN1A, RB1, CDK1, CHEK1, PCNA, CCNB1
Negative regulation of G1/S transition of mitotic cell cycle	2.07E-08	6	CHEK2, CDKN1A, RB1, CDK1, PCNA, CCNB1
DNA damage checkpoint	1.70E-07	6	CHEK2, CDKN1A, CDK1, CHEK1, PCNA, CCNB1
Positive regulation of macromolecule biosynthetic process	1.94E-07	12	CHEK2, FOXM1, CCNE1, RB1, PARP1, TSC2, EEF2, RPS6KB1, CDK1, CHEK1, PCNA, SRC
DNA repair	3.08E-07	8	CHEK2, FOXM1, MSH6, PARP1, CDK1, CHEK1, PCNA, XRCC1
Positive regulation of gene expression	3.21E-07	12	CHEK2, FOXM1, CCNE1, RB1, PARP1, TSC2, EEF2, RPS6KB1, CDK1, CHEK1, SRC, CCNB1
Positive regulation of cell cycle arrest	3.74E-07	5	CHEK2, CDKN1A, CDK1, PCNA, CCNB1
Positive regulation of cellular biosynthetic process	3.74E-07	12	CHEK2, FOXM1, CCNE1, RB1, PARP1, TSC2, EEF2, RPS6KB1, CDK1, CHEK1, PCNA, SRC
Pathway			
Cell cycle	1.06E-09	8	CHEK2, CCNE1, CDKN1A, RB1, CDK1, CHEK1, PCNA, CCNB1
p53 signaling pathway	1.06E-09	7	CHEK2, CCNE1, CDKN1A, TSC2, CDK1, CHEK1, CCNB1
FOXM1 transcription factor network	1.61E-09	6	CHEK2, FOXM1, RB1, CDK1, CCNB1, XRCC1
E2F mediated regulation of DNA replication	9.24E-08	5	CCNE1, RB1, CDK1, PCNA, CCNB1
mTOR signaling pathway	9.46E-07	5	CCNE1, TSC2, EEF2, RPS6KB1, EIF4EBP1
ATM signaling pathway	4.84E-05	3	CHEK2, CDKN1A, CHEK1
DNA double-strand break repair	5.32E-05	5	CHEK2, PARP1, CHEK1, PCNA, XRCC1
ErbB signaling pathway	8.14E-05	4	CDKN1A, RPS6KB1, EIF4EBP1, SRC
Endocrine resistance	1.16E-04	4	CDKN1A, RB1, RPS6KB1, SRC
HIF-1 signaling pathway	1.39E-04	4	CDKN1A, RPS6KB1, EIF4EBP1, TFRC
Base excision repair	1.57E-04	3	PARP1, PCNA, XRCC1
AMPK signaling pathway	2.39E-04	4	TSC2, EEF2, RPS6KB1, EIF4EBP1
PI3K-Akt signaling pathway	7.78E-04	5	CCNE1, CDKN1A, TSC2, RPS6KB1, EIF4EBP1
Mismatch repair	1.42E-03	2	MSH6, PCNA

CCNF, cyclin F. P-values were corrected for multiple comparisons using the false discovery rate (FDR) (Benjamini and Hochberg).

reported that melanoma cells are able to upregulate transferrin receptor 1 through the hyaluronan/CD44 pathway. It is possible that this pathway promotes proliferation providing alternative iron supply for melanoma cells. High expression of TFRC is

Table VII. Expression of proteins which are negatively correlated with RRM2.

		$RRM2^{downregulated}$	$RRM2^{upregulated}$		
		RPPA (z-score)			
Protein	Gene	Upregulated	Downregulated	P-value	Significance
14-3-3_ζ	YWHAZ	0.0436	-0.0176	0.1293	NS
α-catenin	CTNNB1	0.0676	-0.0037	0.0957	NS
AMPK_α	PRKAA1	0.0076	-0.0467	0.0311	*
Bcl-2	BCL2	0.1080	-0.1967	<0.0001	****
cIAP	BIRC2	0.0042	-0.0600	0.0043	**
E-cadherin	CDH1	0.1282	-0.4331	0.0003	***
ER-α	ESR1	0.0796	-0.0194	0.0013	**
FOXO3a	FOXO3	0.0664	-0.0035	0.0041	**
GATA3	GATA3	0.0059	-0.0189	0.0886	NS
HER2	ERBB2	0.0414	-0.0487	0.0298	*
HER3	ERBB3	0.0900	-0.1863	<0.0001	****
INPP4B	INPP4B	0.0749	-0.0261	0.0007	***
JAB1	COPS5	0.0083	-0.0772	0.0957	NS
JNK2	MAPK9	0.0047	-0.0109	0.6772	NS
p27_pT198	CDKN1B	0.0017	-0.0069	0.7788	NS
p38_MAPK	MAPK14	0.0349	-0.0488	0.0165	*
p38_pT180_Y182	MAPK14	0.0086	-0.0314	0.4440	NS
PARP_cleaved	PARP1	0.0073	-0.0035	0.4065	NS
PDCD4	PDCD4	0.1225	-0.1818	0.0001	***
PDK1	PDPK1	0.0294	-0.0014	0.1348	NS
PDK1_pS241	PDPK1	0.0071	-0.0442	0.2341	NS
PI3K-p85	PIK3R1	0.0116	-0.0613	0.0605	NS
PKC-α	PRKCA	0.0155	-0.0969	0.0414	*
PKC-α_pS657	PRKCA	0.0906	-0.0247	0.0307	*
PRDX1	PRDX1	0.0146	-0.0238	0.5525	NS
PREX1	PREX1	0.0619	-0.0082	0.2623	NS
Rab25	RAB25	0.0707	-0.1177	<0.0001	****
Rad50	RAD50	0.0570	-0.0174	0.0624	NS
Src	SRC	0.0459	-0.0729	0.0033	**
Src_pY527	SRC	0.0315	-0.0300	0.3020	NS
VEGFR2	KDR	0.0191	-0.0239	0.4077	NS
YAP	YAP1	0.0412	-0.0283	0.0215	*
YAP_pS127	YAP1	0.1242	-0.0491	0.0106	*

RRM2, ribonucleotide reductase family member 2; RPPA, reverse-phase protein array. ****, extremely significant (P<0.0001); ***, extremely significant (P=0.0001 to 0.001); **, very significant (P=0.001 to 0.01); *, significant (P=0.01 to 0.05); NS, not significant (P≥0.05).

associated with unfavorable prognosis in breast and pancreatic cancer (52-54).

This newly discovered relationship between mRNA expression of CCNF and RRM2 provide and attractive point for further investigations in the field of dermato-oncology. Our analysis was performed using independent data obtained from TCGA and provide many key results that can be used in further explanation of the precise mechanisms. Moreover, we expect that the present results will be useful to other researchers and induce further investigations, essential for better diagnosis, prediction, therapy response, but also for better selection of patients for optimal therapy against skin melanoma. A high number of clones contributes to an exceptional level of intratumor heterogeneity of melanoma, but also refers to metastases which may originate from different subclones of the primary tumor. This creates an obstacle to proper diagnosis and successful treatment (55). Increased research on the topic is needed for understanding the limitation or failure of contemporary therapies and the precise mechanism must and will be elucidated by our team *in vitro* in the immediate future using melanoma cancer cell panels. We suggest here to investigate the precise mechanism indicated in the study using all following cell lines: SK-MEL-1, A375, G-361, SK-MEL-3, SH-4, SK-MEL-24, RPMI-7951. However, we hope that the publication of *in silico* analyses accelerates

Table VIII. Biological process and pathway analysis of genes whose products are negatively correlated with RRM2 expression.

Factor	P-value	Number of genes	Gene list
Biological process			
Regulation of cell proliferation	4.03E-09	13	FOXO3, CDH1, BIRC2, PRKCA, YAP1, ERBB2, ERBB3, ESR1, BCL2, RAB25, MAPK14, PRKAA1, SRC
Apoptotic process	1.03E-08	13	FOXO3, CDH1, BIRC2, PRKCA, YAP1, ERBB2, ERBB3, PDCD4, ESR1, BCL2, MAPK14, PRKAA1, SRC
Regulation of apoptotic process	1.39E-08	12	FOXO3, CDH1, BIRC2, PRKCA, YAP1, ERBB2, ERBB3, PDCD4, ESR1, BCL2, PRKAA1, SRC
Negative regulation of signal transduction	2.30E-08	11	FOXO3, CDH1, PRKCA, YAP1, ERBB3, PDCD4, ESR1, BCL2, MAPK14, PRKAA1, SRC
Negative regulation of apoptotic process	8.07E-07	9	BIRC2, PRKCA, YAP1, ERBB2, ERBB3, PDCD4, BCL2, PRKAA1, SRC
Regulation of intracellular signal transduction	8.07E-07	11	BIRC2, PRKCA, ERBB2, ERBB3, PDCD4, ESR1, BCL2, INPP4B, MAPK14, PRKAA1, SRC
Positive regulation of intracellular signal transduction	1.09E-06	9	BIRC2, PRKCA, ERBB2, ERBB3, ESR1, BCL2, MAPK14, PRKAA1, SRC
Regulation of cell motility	4.00E-06	8	CDH1, PRKCA, ERBB2, ERBB3, BCL2, RAB25, MAPK14, SRC
Positive regulation of protein modification process	5.45E-06	9	BIRC2, PRKCA, ERBB2, ERBB3, ESR1, BCL2, MAPK14, PRKAA1, SRC
Regulation of cellular component movement	6.29E-06	8	CDH1, PRKCA, ERBB2, ERBB3, BCL2, RAB25, MAPK14, SRC
MAPK cascade	8.78E-06	8	PRKCA, ERBB2, ERBB3, PDCD4, ESR1, MAPK14, PRKAA1, SRC
Positive regulation of protein phosphorylation	1.03E-05	8	PRKCA, ERBB2, ERBB3, ESR1, BCL2, MAPK14, PRKAA1, SRC
Signal transduction by protein phosphorylation	1.03E-05	8	PRKCA, ERBB2, ERBB3, PDCD4, ESR1, MAPK14, PRKAA1, SRC
Regulation of canonical Wnt signaling pathway	3.05E-05	5	FOXO3, CDH1, YAP1, MAPK14, SRC
Pathway			
EGFR tyrosine kinase inhibitor resistance	1.74E-07	6	FOXO3, PRKCA, ERBB2, ERBB3, BCL2, SRC
Proteoglycans in cancer	5.39E-07	7	PRKCA, ERBB2, ERBB3, PDCD4, ESR1, MAPK14, SRC
a6b1 and a6b4 Integrin signaling	9.50E-06	4	CDH1, PRKCA, ERBB2, ERBB3
Endocrine resistance	1.17E-05	5	ERBB2, ESR1, BCL2, MAPK14, SRC
Signaling by ERBB2	4.47E-05	4	PRKCA, ERBB2, ERBB3, SRC
Focal adhesion	2.06E-04	5	BIRC2, PRKCA, ERBB2, BCL2, SRC
ErbB signaling pathway	2.06E-04	4	PRKCA, ERBB2, ERBB3, SRC
NGF signalling via TRKA from the plasma membrane	2.42E-04	6	FOXO3, PRKCA, ERBB2, ERBB3, MAPK14, SRC
FAS (CD95) signaling pathway	4.38E-04	3	BIRC2, MAPK14, SRC
Signalling by NGF	4.82E-04	6	FOXO3, PRKCA, ERBB2, ERBB3, MAPK14, SRC
PI3K/AKT activation	4.82E-04	4	FOXO3, ERBB2, ERBB3, SRC
Cadherin signaling pathway	6.77E-04	4	CDH1, ERBB2, ERBB3, SRC
Pathways in cancer	1.04E-03	5	CDH1, BIRC2, PRKCA, ERBB2, BCL2
Signaling by SCF-KIT	6.93E-04	5	FOXO3, PRKCA, ERBB2, ERBB3, SRC

RRM2, ribonucleotide reductase family member 2. P-values were corrected for multiple comparisons using the false discovery rate (FDR) (Benjamini and Hochberg).

Table IX. Expression of proteins which are positively correlated with RRM2.

		RRM2^{downregulated}	RRM2^{upregulated}		
		RPPA (z-score)			
Protein	Gene	Downregulated	Upregulated	P-value	Significance
4E-BP1	EIF4EBP1	-0.0824	0.0995	0.0010	***
4E-BP1_pS65	EIF4EBP1	-0.0413	0.0554	0.0126	*
4E-BP1_pT70	EIF4EBP1	-0.0152	0.0185	0.2779	NS
ACC_pS79	ACACA	-0.0209	0.0587	0.0782	NS
ACC1	ACACA	-0.0340	0.1352	0.0024	**
Bax	BAX	-0.0251	0.0043	0.8262	NS
C-Raf	RAF1	-0.0244	0.0222	0.0089	**
CDK1_pY15	CDK1	-0.0711	0.0147	0.1638	NS
Chk1	CHEK1	-0.0460	0.0737	<0.0001	****
Chk1_pS345	CHEK1	-0.0142	0.0205	0.0618	NS
Chk2	CHEK2	-0.0412	0.0977	0.0002	***
Cyclin_B1	CCNB1	-0.2434	0.2391	<0.0001	****
Cyclin_E1	CCNE1	-0.0330	0.0445	0.1473	NS
eEF2	EEF2	-0.0765	0.0339	0.1272	NS
EGFR_pY1173	EGFR	-0.0088	0.0283	0.1524	NS
eIF4E	EIF4E	-0.0487	0.0033	0.1951	NS
FoxM1	FOXM1	-0.0608	0.1823	<0.0001	****
GAPDH	GAPDH	-0.0538	0.0416	0.0996	NS
HER3_pY1289	ERBB3	-0.0062	0.0066	0.3676	NS
MSH2	MSH2	-0.0493	0.0314	0.0703	NS
MSH6	MSH6	-0.1812	0.0677	<0.0001	****
Myosin-IIa	MYH9	-0.0317	0.0371	0.4099	NS
NF2	NF2	-0.0205	0.0258	0.2552	NS
p21	CDKN1A	-0.0880	0.1049	0.0025	**
p62-LCK-ligand	SQSTM1	-0.0850	0.0313	0.1070	NS
p90RSK	RPS6KA1	-0.0133	0.0750	0.0363	*
PCNA	PCNA	-0.0499	0.0905	0.0001	***
PRAS40_pT246	AKT1S1	-0.0225	0.0124	0.3327	NS
Rb_pS807_S811	RB1	-0.1222	0.0913	0.0035	**
S6_pS235_S236	RPS6KB1	-0.0110	0.2044	0.0053	**
S6_pS240_S244	RPS6KB1	-0.0516	0.1676	0.0024	**
SLC1A5	SLC1A5	-0.0858	0.0421	0.0743	NS
Src_pY416	SRC	-0.0307	0.0735	0.0630	NS
TFRC	TFRC	-0.1404	0.2463	0.0007	***
Transglutaminase	TGM1	-0.0275	0.0094	0.5674	NS
TSC1	TSC1	-0.0611	0.0051	0.1200	NS

RRM2, ribonucleotide reductase family member 2; RPPA, reverse-phase protein array. ****, extremely significant (P<0.0001); ***, extremely significant (P=0.0001 to 0.001); **, very significant (P=0.001 to 0.01); *, significant (P=0.01 to 0.05); NS, not significant (P≥0.05).

the development and inspires other scientific teams to conduct similar research in the field.

In conclusion, the present study is a first attempt to elucidate the influence of cyclin F mRNA expression on the outcome of melanoma patients. High expression of cyclin F mRNA is associated with worse overall survival. Moreover, *in silico* analysis revealed that upregulated cyclin F mRNA expression is associated with activation of molecular pathways responsible for melanoma proliferation, metastatic potential and survival. These findings are a good starting point to address new cyclin F targets and interactions which drive the increased aggressiveness of the tumor.

Acknowledgements

Not applicable.

Table X. Biological process and pathway analysis of genes whose products are positively correlated with RRM2 expression.

Factor	P-value	Number of genes	Gene list
Biological process			
Cell cycle phase transition	1.99E-08	9	CHEK2, FOXM1, CDKN1A, RB1, RPS6KB1, CHEK1, PCNA, EIF4EBP1, CCNB1
Cell cycle G1/S phase transition	9.08E-08	7	CHEK2, CDKN1A, RB1, RPS6KB1, PCNA, EIF4EBP1, CCNB1
Negative regulation of cell cycle phase transition	1.97E-07	6	CHEK2, CDKN1A, RB1, CHEK1, PCNA, CCNB1
Cell cycle	1.97E-07	11	CHEK2, FOXM1, CDKN1A, RB1, MSH6, RPS6KA1, RPS6KB1, CHEK1, PCNA, EIF4EBP1, CCNB1
Positive regulation of cell cycle	2.96E-07	7	CHEK2, CDKN1A, RB1, RPS6KB1, PCNA, EIF4EBP1, CCNB1
Cell cycle process	3.83E-07	10	CHEK2, FOXM1, CDKN1A, RB1, MSH6, RPS6KB1, CHEK1, PCNA, EIF4EBP1, CCNB1
Regulation of cell cycle	5.63E-07	9	CHEK2, FOXM1, CDKN1A, RB1, RPS6KB1, CHEK1, PCNA, EIF4EBP1, CCNB1
Negative regulation of cell cycle G1/S phase transition	5.94E-07	5	CHEK2, CDKN1A, RB1, PCNA, CCNB1
Regulation of cell cycle arrest	7.05E-07	5	CHEK2, FOXM1, CDKN1A, PCNA, CCNB1
Signal transduction by p53 class mediator	1.11E-06	6	CHEK2, FOXM1, CDKN1A, CHEK1, PCNA, CCNB1
Signal transduction in response to DNA damage	1.11E-06	5	CHEK2, FOXM1, CDKN1A, PCNA, CCNB1
DNA integrity checkpoint	3.58E-06	5	CHEK2, CDKN1A, CHEK1, PCNA, CCNB1
Regulation of cell proliferation	1.36E-04	8	FOXM1, CDKN1A, RB1, RAF1, RPS6KB1, CHEK1, CCNB1, TFRC
Regulation of cell growth	2.26E-04	5	FOXM1, CDKN1A, RB1, RPS6KA1, TFRC
Pathway			
Cell cycle	6.87E-07	6	CHEK2, CDKN1A, RB1, CHEK1, PCNA, CCNB1
Insulin signalling	9.14E-06	4	RAF1, RPS6KA1, RPS6KB1, EIF4EBP1
FOXM1 transcription factor network	9.14E-06	4	CHEK2, FOXM1, RB1, CCNB1
mTOR signaling pathway	5.69E-05	4	RAF1, RPS6KA1, RPS6KB1, EIF4EBP1
p53 signaling pathway	6.70E-05	4	CHEK2, CDKN1A, CHEK1, CCNB1
ATM signaling pathway	9.01E-05	3	CHEK2, CDKN1A, CHEK1
ErbB signaling pathway	1.04E-04	4	CDKN1A, RAF1, RPS6KB1, EIF4EBP1
HIF-1 signaling pathway	1.53E-04	4	CDKN1A, RPS6KB1, EIF4EBP1, TFRC
E2F mediated regulation of DNA replication	2.21E-04	3	RB1, PCNA, CCNB1
G2/M DNA damage checkpoint	2.21E-04	2	CHEK1, CCNB1
G1/S Transition	2.34E-04	4	CDKN1A, RB1, PCNA, CCNB1
EGFR tyrosine kinase inhibitor resistance	1.20E-03	3	RAF1, RPS6KB1, EIF4EBP1
RB tumor suppressor/checkpoint signaling in response to DNA damage	1.25E-03	2	RB1, CHEK1
MAPKinase signaling pathway	1.49E-03	3	RAF1, RPS6KA1, RPS6KB1

RRM2, ribonucleotide reductase family member 2. P-values were corrected for multiple comparisons using the false discovery rate (FDR (Benjamini and Hochberg).

Funding

This study was supported by a grant from the National Science Centre, Poland (grant no. 2016/21/B/NZ7/01121 to AG).

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

MG and AG designed the study. MG and AK performed the analyses, interpreted the data and wrote the study. DG and AG revised manuscript critically for important intellectual content. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Bioethics Committee of the Nicolaus Copernicus University in Toruń functioning at Collegium Medicum in Bydgoszcz (KB 554/2016).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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