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REVIEW

Eukaryotic initiation factor 4E-binding protein 1 (4E-BP1): a master regulator of mRNA translation involved in tumorigenesis

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Protein synthesis activity is abnormally enhanced in cancer cells to support their uncontrolled growth. However, this process needs to be tightly restricted under metabolic stress - a condition often found within the tumor microenvironment - to preserve cell viability. mTORC1 is critical to link protein synthesis activity to nutrient and oxygen levels, in part by controlling the 4E-BP1-eIF4E axis. Whereas mTORC1 and eIF4E are known pro-tumorigenic factors, whose expression or activity is increased in numerous cancers, the role of 4E-BP1 in cancer is not yet definitive. On the one hand, 4E-BP1 has tumor suppressor activity by inhibiting eIF4E and, thus, blocking mRNA translation and proliferation. This is corroborated by elevated levels of phosphorylated and hence inactive 4E-BP1, which are detected in various cancers. On the other hand, 4E-BP1 has pro-tumorigenic functions as it promotes tumor adaptation to metabolic and genotoxic stress by selectively enhancing or preventing the translation of specific transcripts. Here we describe the molecular and cellular functions of 4E-BP1 and highlight the distinct roles of 4E-BP1 in cancer depending on the microenvironmental context of the tumor.

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INTRODUCTION

Cancer cells reprogram their proteome in order to proliferate and adapt to the stressful tumor microenvironment. It is therefore not surprising that deregulations in expression and activity of translational regulators are often found in tumors. Frequently, overexpression of translation factors has been described in cancers to uncouple protein synthesis from inhibition by tumor cell stresses,[1] which is consistent with the findings that elevated protein synthesis is a common feature in the vast majority of malignancies.[2] In addition to these quantitative changes in overall protein synthesis, qualitative changes by selective translation of a subset of mRNAs modifies the proteome and favors neoplastic growth.[2]

Mammalian target of rapamycin (mTOR), functionally assembling to mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) with a number of other proteins, is a central mediator of translational control, whose activation is influenced by several intra- and extracellular stimuli (see 4E-BP1 in cellular signaling pathways). mTORC1 essentially adapts mRNA translation rates by regulating the activity of its main downstream effectors: eukaryotic initiation factor 4E-binding protein 1 (EIF4EBP1, better known as 4E-BP1) and ribosomal protein S6 kinase 1 (S6K1).[3] In addition, ribosomal protein S6 kinase 2 (S6K2), IGF2 mRNA-binding protein 2 (IMP2) and La-related protein 1 (LARP1) were also shown to be direct mTORC1 targets and to be synergistically implicated in the regulation of mRNA translation.[4–6] In cancer, oncogenic activation of mTORC1 signaling because of alterations in signaling pathways upstream of mTORC1 leads to an increase of overall and selective mRNA translation.[3] However, tumors often grow in hostile environments due to defective tumor vasculature or genotoxic or oxidative stress induced by rapid cell division or therapy.[7] As mRNA translation is one of the most energy consuming cellular processes, mTORC1 activity has to be blocked to preserve energy and generate an adaptive response, which combines to maintain cell survival.[8] Two of the most important cancer-promoting translational regulators, mTORC1 and eukaryotic initiation factor 4E (eIF4E), have been thoroughly investigated. However, the role of 4E-BP1 that is directly regulated by mTORC1 and directly regulates eIF4E in cancer is not well understood and is therefore the focus of this review.

4E-BP1 belongs to a family of eIF4E-binding proteins (4E-BP1, −2 and −3), each of which is encoded by a distinct gene.[4] Nonetheless, they show a high degree of homology to each other.[4,9,10] Among these family members, however, deregulations in 4E-BP1 expression or activation are reported far more frequently in a wide range of cancer entities compared with deregulations of 4E-BP2/3 (Table 1). The 4E-BP1 protein structure is given in Figure 1.[9,10] Non-phosphorylated and thus active 4E-BP1 inhibits cap-dependent translation initiation by binding eukaryotic translation initiation factor 4E (eIF4E) and prohibiting the formation of the 48S pre-initiation complex.[11,12]

At least nine initiation factors are required in this process. Among them is eIF4F, which itself is composed of three initiation factors: eIF4G, eIF4A and eIF4E. eIF4E is the cap-binding protein, associating with 5´cap, while eIF4A functions as an RNA-helicase.[13] eIF4G has the role of a scaffold protein and associates with eIF4A, eIF4E, poly-A-binding protein (PABP) and eIF3, thereby linking mRNA, eIF4F and the ribosomal 40S subunit to each other.[13]

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Table 1. Selected publications on 4E-BP1 dysregulations in most reported cancer entities

	4E-BP1 alteration reported	*eIF4E alteration reported*	*Outcome reported*	*Pathomechanism of 4E-BP1 dysregulation reported*	*References*
Acute lymphatic leukemia	Significantly elevated levels of p-4E-BP1	Not reported	Positively correlated with poor prognosis at diagnosis and relapse	Hyperactivation of mTORC1 signaling	Nemes *et al.*[86]
Acute myeloid leukemia	Positive staining for p-4E-BP1 in 77% of examined samples	High eIF4E levels		Mutation/activation of FLT3 receptor; hyperactivation of PI3K/Akt/mTORC1 signaling	Chen *et al.*[87]
Adrenocortical tumors	Positive staining for total 4E-BP1 in all tumor samples evaluated (except of one ACC); this staining was intermediate to high in 75% of ACCs and in all ACA evaluated; staining for p-4E-BP1 was positive in 95% of ACCs and in all ACAs; this staining was intermediate to high in 60% of ACCs and in all ACAs	Not reported		Hyperactivation of mTORC1 signaling	De Martino *et al.*[88]
Ameloblastoma	Positive staining for cytoplasmic p-4E-BP1 in 64.7% and for nuclear p-4E-BP1 in 27.1% of examined tumors	Not reported	Expression of p-4E-BP1 in the nucleus related to invasiveness		Li *et al.*[89]
Angiosarcoma	Positive staining for p-4E-BP1 in 88% of samples; 55.2% exhibited high, 44.8% exhibited low intensity staining; an average of 54% of tumor cells expressed p-4E-BP1; weak focal expression of p-4E-BP1 in 25% of normal vasculature	Positive staining for eIF4E in 87% of samples; in 60.3% high intensity and 39.7% low intensity of eIF4E staining; on an average, 70% of tumor cells expressed eIF4E; weak focal expression of eIF4E in 36.4% of normal vasculature specimens	Higher p-4E-BP1 levels in metastatic lesions; higher numbers of p-4E-BP1 positively stained tumor cells in samples originating from radiation-induced AS, compared with sporadic AS; higher numbers of eIF4E positively stained tumor cells in samples originating from radiation-induced AS compared with sporadic AS		Lahat *et al.*[90]
	Positive staining for p-4E-BP1 in 42% of tumors	Not reported			Italiano *et al.*[91]
Astrocytoma	Positive staining for p-4E-BP1 compared with normal tissue	Not reported	p-4E-BP1 expression positively correlates with tumor grade, increased angiogenesis; p-4E-BP1 positivity predicts shortened overall survival	Hyperactivation of mTORC1 signaling	Korkolopoulou *et al.*[92]
Bladder, urothelial carcinoma	Positive cytoplasmic staining for 4E-BP1 in 68% of examined tumors	Not reported	4E-BP1 expression positively correlates with pathological stage (pT); 4E-BP1 expression positively correlates with divergent aggressive histology and invasion	Downregulation of mTORC1 pathway	Schultz *et al.*[93]
			High p-4E-BP1 expression inversely correlates with recurrence-free survival time		Nishikawa *et al.*[94]
	Expression of 4E-BP1 in 95% of tumors; expression of p-4E-BP1 in 100% of tumors	Not reported	Patients with tumor recurrence had lower proportions of tumors with high p-4E-BP1 (51% vs 69%)		Chaux *et al.*[95]
		eIF4E expression elevated in bladder tumors relative to normal bladder tissue	In superficial tumors, high levels of eIF4E expression are associated with poor prognosis and reduced progression-free survival; eIF4E expression highest in invasive cancers	eIF4E expression correlates with increased VEGF expression	De Benedetti and Graff[53]
Breast cancer	Positive staining of p-4E-BP1 in 87.3% of examined cases; overexpression of p-4E-BP1 in 59.2% of breast tumors	Not reported	p-4E-BP1 expression positively correlates with tumor size, histological grade, presence of lymph node metastasis and locoregional recurrence	Hyperactivation PI3K/Akt/mTORC1 signaling; HER2 overexpression	Rojo *et al.*[96]
	Phosphorylation level of 4E-BP1 increases from benign to malign phenotypes; 41.2% of invasive breast carcinoma exhibit high levels of p-4E-BP1 staining	Not reported	Patients with lower 4E-BP1 phosphorylation tended to have longer disease-free survival	Hyperactivation PI3K/Akt/mTORC1 signaling; ErbB2 overexpression	Zhou *et al.*[97]
	High 4E-BP1 mRNA expression	Not reported	High 4E-BP1 mRNA expression positively correlates with worse outcome; 4E-BP1 mRNA expression is an independent prognostic factor	(11q13;) 8p12 (co-) amplification	Karlsson *et al.*[98]
	High 4E-BP1 mRNA expression; Differences in p-4E-BP1/4E-BP1 ratio and localization (cytoplasm or nucleus)	Not reported	4E-BP1 mRNA expression positively correlates with poor outcome; several correlations between p-4E-BP1, 4E-BP1 protein expression and different outcome variables have been shown	Hyperactivation PI3K/Akt/mTORC1 signaling	Karlsson *et al.*[99]
		3–30-fold increased eIF4E expression compared with normal and benign tissues	⩾Sevenfold elevation in eIF4E expression is a strong, independent prognostic indicator of recurrence and death; patients with ⩾14-fold elevated eIF4E expression had a 7.2-fold greater risk of cancer recurrence	Stimulation of eIF4E expression by hypoxia or c-Myc	De Benedetti and Graff[53]
Cholangiocarcinoma	High positive staining for p-4E-BP1 in 50.6% of cases	Not reported	p-4E-BP1 expression is a predictor for poor prognosis in ICC patients; p-4E-BP1 is an independent prognostic factor in ICC	Hyperactivation of mTORC1 signaling	Wang *et al.*[100]
	Meta-analysis regarding p-4E-BP1	Not reported	p-4E-BP1 is a prognostic biomarker for overall survival		Ruys *et al.*[101]
Colorectal carcinoma; colorectal adenomas with high-grade intraepithelial neoplasia	Phosphorylation level of 4E-BP1 increases from benign to malign phenotypes; significant overexpression of p-4E-BP1 in cancerous lesions and adenomas with high-grade intraepithelial neoplasia, compared with normal, non-cancerous tissue and adenomas with low-grade	Not reported	High p-4E-BP1 expression positively correlated with depth of infiltration	Hyperactivation of mTORC1 signaling	Zhang *et al.*[102]

Table 1. (Continued)

	4E-BP1 alteration reported	*eIF4E alteration reported*	*Outcome reported*	*Pathomechanism of 4E-BP1 dysregulation reported*	*References*
	intraepithelial neoplasia; positive staining for p-4E-BP1 in 82.14% of cancerous lesions				
	Significantly elevated levels of 4E-BP1 in cancerous colon and rectum tissue compared with normal tissue	Not reported	4E-BP1 expression positively correlates with lymph node invasion and stage; positive staining for 4E-BP1 inversely correlates with survival time and time to recurrence		Chao *et al.*[103]
		eIF4E expression significantly elevated in colorectal carcinomas relative to normal colonic tissue	Associated with histological type of the lesion: lowest level of eIF4E expression in normal colon tissue, highest level of eIF4E expression in colorectal adenocarcinomas	Upregulation of eIF4E early event in colon carcinogenesis; associated with increased expression of cyclin D1	De Benedetti and Graff;[53] Berkel *et al.*[161]
Endometrial cancer	Significantly elevated levels of p-4E-BP1 compared with benign or grade G1 tissue	Not reported	Elevated levels of p-4E-BP1 in high-grade (grade 3) tumors	Hyperactivation of PI3K/Akt/mTORC1 signaling; HER2 overexpression	Rice *et al.*[104]
	Most tumors positive for cytoplasmic and nuclear p-4E-BP1 staining	Not reported	Nuclear p-4E-BP1 expression significantly increased in carcinomas of poorer differentiation and more frequent in tumors with deep infiltration of the myometrium; p-4E-BP1 expression in high-grade, high-stage tumors	PTEN loss; hyperactivation of PI3K/Akt/mTORC1 signaling	Darb-Esfahani *et al.*[105]
Esophageal cancer	4E-BP1 levels elevated compared with normal tissue; significant dephosphorylation of 4E-BP1 compared with normal tissue; significant higher levels of 4E-BP1/eIF4E complex were found in tumor compared with normal tissue	eIF4E levels elevated compared with normal tissue; no differences in eIF4E phosphorylation between tumor and normal tissue	High levels of 4E-BP1 inversely correlate with stage; high levels of eIF4E positively correlate with stage		Salehi and Mashayekhi[106]
Gastric cancer	Positive staining for 4E-BP1 in 68.5% of tumors; positive staining for p-4E-BP1 in 49.7% of tumors	Not reported	4E-BP1 expression positively correlates with lymph node metastasis and poor differentiation; expression of p-4E-BP1 positively correlates with tumor size, node metastasis and TNM stage; p-4E-BP1 overexpression inversely correlates with median survival time		Jiao *et al.*[107]
	Overexpression of 4E-BP1 mRNA and p-4E-BP1 in cancerous tissues compared with paracancerous and normal tissues (increasing from benign to malign tissue)	Not reported	p-4E-BP1 elevation positively correlates with histological grade, depth of invasion, distant metastasis and lymph node metastasis	Hyperactivation of Akt/mTORC1 signaling via GOLPH3	Peng *et al.*[108]
		eIF4E overexpression in 74% of tumors	Marked eIF4E overexpression associated with vascular invasion; marked eIF4E overexpression associated with significantly poorer survival rate		Chen *et al.*[162]
Gastroenteropancreatic neuroendocrine tumors	Positive cytoplasmic staining for 4E-BP1 in 92.9% of tumors; positive cytoplasmic and nuclear staining of p-4E-BP1 in 79.8% and 68.7% of tumors	Positive staining for p-eIF4E in 79.4% of tumors	p-4E-BP1 (cytoplasmic and nuclear) and 4E-BP1 positively correlate with higher proliferation capacities; higher expression of cytoplasmic p-4E-BP1 compared with primary tumor positively correlates with nodal spread; positive correlation of higher expression of nuclear p-4E-BP1 and distant spread was of borderline significance; patients with cytoplasmic p-4E-BP1 expression had a trend towards longer disease-specific survival than patients without phosphorylation of 4E-BP1; p-eIF4E expression positively correlated with higher proliferative capacity	Hyperactivation of mTORC1 signaling	Kasajima *et al.*[109]
Gastrointestinal cancer (combination cohort of gastric and colorectal cancer)	4E-BP1 expression increased in most of t he gastrointestinal cancers compared with normal tissue; significant dephosphorylation of 4E-BP1 in tumors compared with normal tissue; significant higher levels of the 4E-BP1/eIF4E complex in tumor tissue compared with normal tissue	eIF4E levels significantly higher in tumors compared with normal tissue; no difference in eIF4E phosphorylation in gastric cancers and decrease of eIF4E phosphorylation in colorectal cancers	Inverse correlation between 4E-BP1 elevation and N and M stages; significant higher elevation of 4E-BP1 in node-negative patients and patients without distant metastasis; node-negative cancers showed significant higher elevation of 4E-BP1/eIF4E ratio compared with node-positive cancers		Martín *et al.*[110]
Gliomas, pediatric	Positive staining for p-4E-BP1 in 80% of high-grade and 50% of low-grade gliomas	Not reported	80% of high-grade and 50% of low-grade gliomas showed higher levels of p-4E-BP1 expression: tumor grade correlated positively with expression of p-4E-BP1; p-4E-BP1 expression positively correlates with worse progression-free survival, p-4E-BP1's positive correlation with overall survival in low-grade gliomas maintained borderline significance	PTEN loss, PTEN promotor methylation, hyperactivation of PI3K/Akt/mTORC1 signaling	Mueller *et al.*[111]
Head and neck squamous cell carcinoma	Lower p-4E-BP1 levels in tumors as in non-cancerous tissue measured by western blot higher p-4E-BP1 positive staining in tumor (50%) as in non-cancerous tissue (4.5%) measured by immunohistochemistry		50.0% sensitive and 95.5% specific in differentiating normal mucosa from HNSCC	Hyperactivation of Akt/mTORC1 signaling	Clark *et al.*[112]
	eIF4E:4E-BP1 relation of mRNA expression	eIF4E:4E-BP1 relation of mRNA expression	Elevated eIF4E:4E-BP1 ratio positively correlates with increased disease recurrence in HNSCC	eIF4E gene amplification and overexpression	Sunavala-Dossabhoy *et al.*[113]
		Elevated eIF4E expression in HNSCC compared with benign lesions	Presence of as little as 5% eIF4E-positive stained cells at the surgical margins was sufficient to predict local		De Benedetti and Graff[53]

Table 1. (Continued)

	4E-BP1 alteration reported	*eIF4E alteration reported*	*Outcome reported*	*Pathomechanism of 4E-BP1 dysregulation reported*	*References*
			recurrence in two-thirds of the patients with recurrence within 2 years		
Liposarcoma, myxoid/ round cell	Positive staining for p-4E-BP1 in 60% of all tumors; high levels of p-4E-BP1 more frequently in round cell tumors compared with myxoid tumors (80% vs 57%)	Not reported	Tumors with treatment effect were least likely to demonstrate high levels of p-4E-BP1 (42%); hyperactivation of the PI3K/Akt pathway via activating mutation of PIK3CA, loss of PTEN or IGF1R expression have a role in round cell transformation	Activating mutation of PIK3CA; loss of PTEN; IGF1R expression; hyperactivation PI3K/Akt/mTORC1 signaling	Demicco *et al.*[114]
Lung cancer, small cell and large cell neuroendocrine	More intense positive staining for p-4E-BP1 in high-grade tumors (LCNEC and SCLC) than low-grade tumors; total 4E-BP1 level comparable intensity in all samples, p-4E-BP1 more heterogeneously present, with a higher intensity in LCNEC cases	Not reported	More intense positive staining of p-4E-BP1 in high-grade tumors (LCNECs and SCLCs) than low-grade tumors; p-4E-BP1 is expressed at higher levels in small tumors, with a strong significance in high-grade tumors	Hyperactivation of mTORC1 signaling	Righi *et al.*[115]
	Positive nuclear and/or cytoplasmic staining for p-4E-BP1 in 66% of examined SCLC; weak expression in 13%, moderate expression in 40%, strong expression in 47% of positive SCLC	Not reported	Positive staining for p-4E-BP1 higher in metastatic tumor tissues; high p-4E-BP1 expression inversely correlates with overall survival, especially in patients with limited disease or completed treatment		Roh *et al.*[116]
Lung cancer, non-small cell	Positive staining for p-4E-BP1 in 43% (Thr37/46) and 49% (Thr70) of examined tumors; significantly higher expression of p-4E-BP1 in squamous cell carcinoma compared with adenocarcinoma	Not reported	p-4E-BP1 (Thr70) expression higher in tumors with diameter larger than 3 cm and nodal metastasis; p-4E-BP1 (Thr70) expression inversely correlates with overall survival and is an independent prognostic factor		Lee *et al.*[117]
		eIF4E expression three- to eightfold higher in bronchial adenocarcinomas (but not squamous cell carcinomas) than in normal lung	Associated with histological grade and invasiveness of the tumor		De Benedetti and Graff[53]
Lymphoma, thymic	p-4E-BP1 increased in ATM−/− thymocytes relative to ATM+/+ thymocytes	Not reported		ATM loss	Kuang *et al.*[118]
Lymphoma, non-Hodgkin´s		Elevated eIF4E expression	Elevated eIF4E expression positively correlates with aggressiveness of the lesion		De Benedetti and Graff[53]
Malignant epidermal tumors	Positive staining for p-4E-BP1 in 52% of examined tumors	Not reported	Positive staining for p-4E-BP1 more frequently in malignant tumors than in benign tumors or normal tissue	Hyperactivation of Akt/mTORC1 signaling	Chen *et al.*[119]
Malignant mixed mullerian tumor	Significantly elevated levels of p-4E-BP1 compared with benign or grade G1 tissue	Not reported		Hyperactivation of PI3K/Akt/ mTORC1 signaling	Rice *et al.*[104]
Melanoma	Positive staining for total 4E-BP1 in 97% of examined tumors (on average 95% of cells positive); positive staining for p-4E-BP1 in 74% of examined tumors (on average 54% of cells positive); nucleus and cytoplasm both positive for total 4E-BP1 and p-4E-BP1	Not reported	p-4E-BP1 expression inversely correlates with overall survival and post-recurrence survival	BRAF and PTEN mutation; hyperactivation of MAPK and PI3K signaling	O'Reilly *et al.*[120]
Mesothelioma, diffuse peritoneal	4E-BP1 expression in 100% of tumors; p-4E-BP1 expression in 95% of tumors	Not reported		EGFR and PDGFRB activation; hyperactivation of PI3K/Akt/ mTORC1 and Erk/mTORC1 signaling	Perrone *et al.*[121]
Nasopharyngeal carcinoma	57.4% of examined tumors show high expression, 42.6% show low expression of p-4E-BP1	Not reported	High expression of p-4E-BP1 positively correlates with lymph node metastasis; p-4E-BP1 expression is a prognostic predictor of overall survival in NPC	LMP1 upregulation; hyperactivation of mTORC1 signaling	Chen *et al.*[122]
	Significantly higher expression of p-4E-BP1 in NPC (61.7%) compared with the non-cancerous nasopharyngeal tissue (47.0%)	Not reported	Positive expression of p-4E-BP1 significantly correlated inversely with overall survival rates of NPC patients; positive expression of p-4E-BP1 is an independent poor prognostic factor	Hyperactivation of mTORC1 signaling	Wang *et al.*[123]
Neuroblastoma	Most tumors positive for p-4E-BP1 staining	Not reported	Expression of p-4E-BP1 positively correlated with low differentiation		Iżycka-Świeszewska *et al.*[124]
Ovarian carcinoma	Positive staining for p-4E-BP1 (mainly nuclear) in 41.1% of examined tumors; positive cytoplasmic staining for p-4E-BP1 in 25% of tumors	Not reported	Malignant tumors are more often positive for p-4E-BP1 than benign or borderline tumors; significant positive correlation of p-4E-BP1 expression with histological grade and poor prognosis; cytoplasmic staining only in malignant tumors; cytoplasmic staining positively correlated with histological grade	Hyperactivation of mTORC1 signaling	Castellvi *et al.*[125]
	Positive staining for p-4E-BP1 in 85.4% of tumors	Not reported	p-4E-BP1 overexpression associated with advanced stage, histological grade, residual mass, shorter disease-free survival rate and chemoresistance		No *et al.*[126]

Table 1. (Continued)

	4E-BP1 alteration reported	*eIF4E alteration reported*	*Outcome reported*	*Pathomechanism of 4E-BP1 dysregulation reported*	*References*
Paget´s disease, extra-mammary	Positive staining for p-4E-BP1 in 81.3% of tumors	Not reported	p-4E-BP1 expression positively correlates with invasiveness; invasive specimens and lymph node metastases showed nuclear staining in most of the cells	Hyperactivation of Akt/mTORC1 signaling	Chen *et al.*[127]
Prostate cancer	4E-BP1 and p-4E-BP1 evident in cytoplasm and nucleus of prostate tissues; total cytoplasmic and nuclear 4E-BP1 expression similar in all groups, trend to increased expression across all tissue types; p-4E-BP1 cytoplasmic intensity significantly increased in prostate cancer across tissue types, highest staining in high-grade prostate cancer; cytoplasmic p-4E-BP1 intensity significantly increased in high-grade prostate cancer versus adjacent BPH, low-grade prostate cancer versus adjacent BPH and high-grade prostate cancer versus BPH from patients without prostate cancer; nuclear p-4E-BP1 histoscores significantly increased across all tissue types, highest nuclear histoscores evident in prostate cancer tissues; increased nuclear p-4E-BP1 in high-grade prostate cancer versus BPH from patients without prostate cancer	All prostate tissues similar percent area stained for cytoplasmic eIF4E, but varied in staining intensity; in BPH tissues eIF4E cytoplasmic staining intensity is low, in low-grade prostate cancer tissues cytoplasmic staining intensity is increased and further elevated in high-grade prostate cancer; eIF4E cytoplasmic staining intensity is significantly increased across all tissue types, low-grade prostate cancer versus adjacent BPH, high-grade prostate cancer versus adjacent BPH and high-grade versus low-grade prostate cancer; highest nuclear eIF4E histoscores were evident in prostate cancer tissues relative to BPH tissues; nuclear eIF4E histoscores significantly increased across all tissue groups; significantly increased staining in high-grade prostate cancer versus BPH from patients without prostate cancer	Patients with high cytoplasmic p-4E-BP1 levels were ~2.5 times likelier to die of prostate cancer; fraction of patients with reduced 4E-BP1 expression showed substantially reduced survival compared with prostate cancer patients showing uniform 4E-BP1 expression; patients whose prostate cancer tissues showed uniform 4E-BP1 expression were only ~25% as likely to die of prostate cancer as patients whose tumors showed reduced 4E-BP1 staining; patients with high eIF4E expression were ~2.7 times likelier to die of prostate cancer than patients with low eIF4E expression		Graff *et al.*[128]
	p-4E-BP1 expression (mainly nuclear) significantly higher in prostate cancer compared with BPH and HGPIN	Not reported	p-4E-BP1 expression is correlated with T stage and distant metastases	Hyperactivation of mTORC1 signaling	Dai *et al.*[129]
	Significantly higher expression of 4E-BP1 and p-4E-BP1 in PIN and cancer (increasing) compared with normal glands	eIF4E expression very high in basal cells, PIN and cancer; expression lower for luminal cells, almost half the expression score of PIN		Hyperactivation of PI3K/Akt/mTORC1 signaling	Kremer *et al.*[130]
		eIF4E overexpression	Increased eIF4E expression is specifically associated with advanced disease; eIF4E expression most dramatically upregulated in prostate cancers with a Gleason score >7	eIF4E overexpression is associated with elevated levels of VEGF and higher microvessel density	De Benedetti and Graff[53]
Renal cell carcinoma, clear cell	Levels of 4E-BP1 in metastatic lesions sign. higher than in primary tumor and benign tissue; levels of 4E-BP1 in primary tumors higher than in benign tissue	Not reported	4E-BP1 expression positively correlates with pathological stage and tumor size; 4E-BP1 expression higher in metastatic carcinoma and in primary carcinoma than benign tissues	PTEN loss; hyperactivation of Akt/mTORC1 signaling	Schultz *et al.*[131]
	Highly positive staining of p-4E-BP1 in 86.4% of tumors	Not reported	p-4E-BP1 expression is an independent predictor of recurrence-free survival time		Haddad *et al.*[132]
Renal cell carcinoma, chromophobe	4E-BP1 staining significantly higher in tumor tissues than in normal kidney	Not reported		PTEN loss; dysregulation of mTORC1 signaling	Chaux *et al.*[133]
Rhabdomyosarcoma in childhood	High p-4E-BP1 expression is positively correlated with poor outcome	Not reported	High p-4E-BP1 expression positively correlated with poor overall survival, high p-4E-BP1 expression positively correlated with poor disease-free survival	Hyperactivation of PI3K/Akt/mTORC1 signaling	Petricoin *et al.*[134]
Rhabdomyosarcoma, alveolar and embryonal	Levels of p-4E-BP1 are increased in 62% of alveolar rhabdomyosarcomas and in 71% of embryonal rhabdomyosarcomas	Not reported		Hyperactivation of PI3K/Akt/mTORC1 signaling	Cen *et al.*[135]
Solitary fibrous tumors	Positive staining for p-4E-BP1 (nuclear and cytoplasmic) in 80.3% of tumors	Not reported	Expression of p-4E-BP1 positively correlated with necrosis	Hyperactivation of Akt/mTORC1 signaling	Yamada *et al.*[136]
Thyroid carcinoma, several histological types	Aggressive PTC exhibit higher levels of p-4E-BP1 and 4E-BP1 compared with micropapillary carcinomas, PTC-C or benign thyroid nodules; PTC-C, PTC-A and MTC tumor cells also revealed high levels of p-4E-BP1 proteins	eIF4E expression in 86% of thyroid tumors (primarily cytoplasmic); adjacent normal thyroid tissue was eIF4E negative in all tissue specimens tested	Higher expression of 4E-BP1 and p-4E-BP1 in PTC-A and MTC; strong eIF4E expression correlated with histology and was observed in 66% of thyroid carcinomas; aggressive types of thyroid carcinomas more often strongly express eIF4E; aggressive PTC exhibit higher levels of eIF4E than micropapillary carcinomas, PTC-C or benign thyroid nodules; the level of eIF4E expression positively correlates with the TNM stage of the tumor	Hyperactivation of mTORC1 signaling	Kouvaraki *et al.*[137]

Abbreviations: ACA, adrenocortical adenomas; ACC, adrenocortical carcinoma; AS, angiosarcoma; BPH, benign prostate hyperplasia; eIF4E, eukaryotic initiation factor 4E; ICC, intrahepatic choleangiocarcinoma; HGPIN, high-grade prostate intraepithelial neoplasia; HNSCC, head and neck squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma of the lung; MTC, medullary thyroid carcinoma; mTORC1, mTOR complex 1; NPC, nasopharyngeal carcinoma; PIN, prostate intraepithelial neoplasia; PTC, papillary thyroid carcinoma; PTC-A, aggressive PTC; PTC-C, conventional PTC; SCLC, small cell lung cancer; VEGF,

As 4E-BP1 shares a common eIF4E-binding motif with eIF4G, the eIF4E-binding motifs of either protein compete for the same phylogenetically invariant dorsal surface of eIF4E.[11,12,14] By competing with each other, 4E-BP1 can sterically block the binding of eIF4G to eIF4E and thereby prevent the assembly of the 48S pre-initiation complex and restrict translational activity.[11,12,14] As tumor cells must adapt to different metabolic conditions in their microenvironment, the association rate of the eIF4F complex is tightly regulated. This is essentially mediated by mTORC1, which thus couples the rate of mRNA translation initiation to various extracellular stimuli.[3,8,15–17]

Although mTORC1-dependent phosphorylation sites are conserved in all three proteins, 4E-BP1 and 4E-BP2 are mostly regulated in a similar manner by mTORC1, whereas considerably less is known about the regulation of 4E-BP3.[4] 4E-BP2 additionally undergoes post-translational modification. For instance, asparagine deamidation leads to enhanced 4E-BP2 phosphorylation and inactivation by mTORC1.[4,18,19] As every 4E-BP contains the eIF4E-binding motif, their physiological roles largely overlap, which further underlines their importance in translational control.[10]

As 4E-BP1 inhibits the pro-oncogenic eIF4E and thereby influences overall and selective mRNA translation (see 4E-BP1: regulation of overall and selective mRNA translation), it is mainly described as a tumor suppressor.[20,21] This is consistent with many studies reporting inactivation by hyperphosphorylation of 4E-BP1 in numerous tumor entities (Table 1). However, because tumor cells must adapt to different metabolic conditions in their microenvironment, mRNA translation must also be adapted. In this context 4E-BP1 can support tumor adaptation to stress and therefore may facilitate tumor progression by selectively modulating the translation of specific key transcripts, apart from overall translation rates. This complex dual role of 4E-BP1 in tumor progression, as well as its molecular and cellular functions will be the topic of this review.

4E-BP1 IN CELLULAR SIGNALING PATHWAYS

The activity of 4E-BP1 is directly dependent on upstream signals controlling mTORC1 activity. This implies that 4E-BP1 activity is modulated by various stimuli and stress conditions, such as growth factors, nutrients and oxygen levels.[8,15,17,22] By responding to these signals, 4E-BP1 contributes to adaption of the rate of mRNA translation initiation to the intracellular metabolic status and extracellular stimuli.[3,8,15–17] Here, we only briefly summarize the main signaling pathways influencing mTORC1 activity and 4E-BP1 phosphorylation, as they were extensively reviewed elsewhere.[3,15–17] Figure 2 gives an overview on the mTORC1 signaling pathway and the main modulating stimuli.

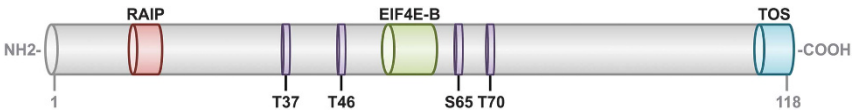

Figure 1. Protein structure and mTORC1-dependent phosphorylation sites of 4E-BP1. 4E-BP1 contains three functional domains: the eIF4E-binding domain,[14] a C-terminal TOR signaling motif (TOS)[173] and an N-terminal RAIP (Arg13, Ala14, Ile15 and Pro16) motif.[174] The TOS and RAIP motif both contribute to the binding of Raptor,[175,176] a scaffold protein of mTOR building a 'bridge' between mTOR and 4E-BP1, necessary for efficient phosphorylation of 4E-BP1.[29,30] mTORC1-dependent phosphorylation of 4E-BP1 proceeds in a hierarchical way: initial phosphorylation of Thr-37 and Thr-46 is followed by Thr-70 and Ser-65,[177,178] whereby phosphorylation of Thr-37 and Thr-46 are thought to be the priming event for subsequent phosphorylation of the C-terminal phosphorylation sites (Thr-70, Ser-65).[177,178]

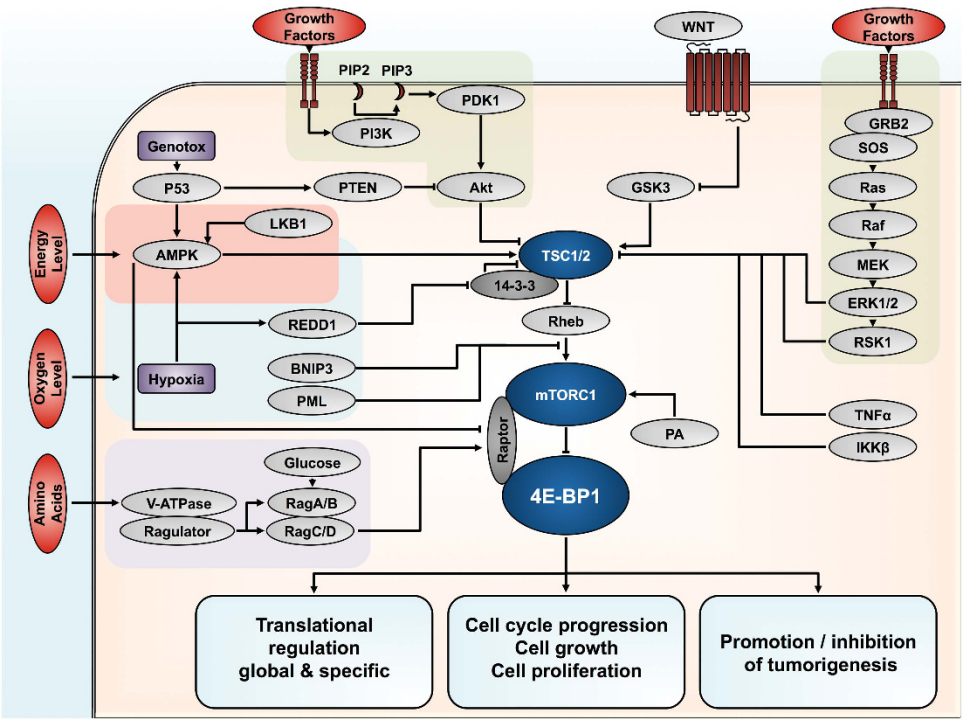

Figure 2. mTORC1 signaling pathway and its upstream effectors regulating the activation status of 4E-BP1. 4E-BP1 activation is directly dependent on mTORC1 kinase activity, which is adjusted by several upstream regulators, mostly important growth factors, amino acids, energy and oxygen levels. Integrating these signals, mTORC1 is a key integrator of microenvironmental signals and regulates global and specific translation rates, cellular proliferation and tumorigenesis via 4E-BP1.

Growth factors

Growth factors are potent stimulators of cellular proliferation. Consistently, signaling pathways associated with growth factor receptors are frequently deregulated in cancer, such as the phosphatidyl-inositol-3-kinase (PI3K) pathway and the MAPK/ERK pathway.[23–25] As both of these pathways signal upstream of mTORC1, their oncogenic activation leads to an overactivation of mTORC1[3,24] (Figure 2). Therefore, growth factor stimulation increases phosphorylation of 4E-BP1 via mTORC1 and enhances translational activity.[11,12,14] As the role of these pathways in cancer has been already reviewed extensively,[23–25] we here refer to these authors (Table 2).

Energy status

Depletion of nutrients or oxygen results in lower cellular energy levels. In order to proliferate, tumor cells have to strictly regulate their energy consumption, as protein synthesis is a highly energy consuming process.[2] An important regulating kinase of nutrient signaling is 5´AMP-regulated kinase (AMPK), which acts as an intracellular energy sensor in response to changes in the cellular AMP:ATP and ADP:ATP ratio.[26] During ATP-depletion, AMPK can block mTORC1 activity by two mechanisms: AMPK is able to activate TSC1/2, a tumor suppressing negative regulator of mTORC1, leading to lower levels of phosphorylated 4E-BP1 and a prohibition of protein synthesis under ATP-lacking conditions.[27] AMPK can also reduce mTORC1 activity by phosphorylating Raptor,[28] a scaffold protein of mTOR building a 'bridge' between mTOR and 4E-BP1, necessary for efficient phosphorylation of 4E-BP1.[29,30] Additionally, liver kinase B1 (LKB1) was shown to be an upstream regulator of AMPK, required for the activation of AMPK and thereby inhibiting mTORC1 pathway in response to nutrient starvation.[31–33] Conversely, increased mTORC1 activity mediates enhanced mitochondrial activity and biogenesis by selectively promoting translation of nucleus-encoded mitochondria-related mRNAs by inactivation of 4E-BPs, which engenders an increase in ATP production capacity.[34] Thereby mTORC1 is able to couple the increase of ATP consumption due to increased protein synthesis to an increase in ATP production.[34]

Oxygen levels

mTORC1 activity is inhibited by hypoxia through multiple independent mechanisms.[35–40] Hypoxia decreases the ADP:ATP ratio, in turn inducing AMPK to block mTORC1 signaling. In addition, by enhancing regulated in development and DNA damage response 1 (*REDD1*) transcription,[35–37] hypoxia leads to lower mTORC1 activity by releasing TSC2 from its growth factor induced binding to 14-3-3 proteins.[38] Finally, promyelocytic leukemia (PML) tumor suppressor and BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP3) were shown to inhibit the association of Rheb and mTORC1 under low oxygen conditions.[39,40] All these mechanisms converge in decreasing the level of phosphorylated (that is, inactive) 4E-BP1 in response to hypoxia.

Table 2. Proto-oncogenes and tumor suppressors affecting the regulation of translation rates by 4E-BP1

	References
Proto-oncogenes	
PI3K	Thorpe *et al.*,[23] Shaw and Cantley,[24] Samuels *et al.*,[141] Samuels *et al.*[140] and Luo *et al.*[142]
Akt	Altomare and Testa[143]
Ras	Dhillon *et al.*[25]
Raf	Dhillon *et al.*[25]
Rheb	Jiang and Vogt[144]
eIF4E	De Benedetti and Graff[53] and Mamane *et al.*[145]
Tumor suppressors	
PTEN	Milella *et al.*[146]
LKB1	Hezel and Bardeesy[147]
TSC1/2	van Veelen *et al.*[138]
Not defined	
AMPK	Faubert *et al.*[139]

Amino acids

Amino acids are a prerequisite for protein synthesis during cellular proliferation and therefore are strong stimuli of mTORC1 activity. Amino acids accumulate in the lysosomal lumen and initiate signaling in a vacuolar H+-adenosine triphosphate ATPase (v-ATPase)-dependent mechanism[41] by which mTORC1 is recruited to the lysosomal membrane, where Rheb as a positive regulator of mTORC1 is located.[42–44] Therefore, the stimulation of nutrients, especially of amino acids, can be seen as a prerequisite for mTORC1 activation by growth factors.

Other signals

Several other stimuli, such as genotoxic stress, Wnt stimulation, inflammation, phosphatidic acid (PA) and glucose have an influence on mTORC1 activity as well.[45–51] Genotoxic stress and the resulting DNA damage increase p53 activity, which leads to an inhibition of mTORC1 signaling via activation of AMPK and enhanced expression of PTEN.[45,46] Stimulation of the Wnt pathway increases mTORC1 activity via inhibition of glycogen synthase kinase 3 (GSK3).[47] Inflammation can enhance mTORC1 signaling via pro-inflammatory cytokines, such as TNFα or IκB kinase-β (IKKβ). Both inactivate TSC2 by direct interaction.[48] Finally, PA increases the activity of mTOR by facilitating the assembly and stabilizing the mTORC1 and mTORC2 complexes.[49,50] Efeyan *et al.* also showed that glucose can recruit mTORC1 to the lysosmal membrane via Rag GTPases in order to activate mTORC1.[51]

4E-BP1: REGULATION OF OVERALL AND SELECTIVE MRNA TRANSLATION

4E-BP1 was discovered as an interactor and inhibitor of the translation initiation factor eIF4E and therefore characterized as a repressor of overall mRNA translation.[52] However, during past years it became increasingly clear that 4E-BP1 exerts selectivity in its ability to restrain translation through preferential blockage of the translation of specific transcripts.[53] More surprisingly, 4E-BP1 may induce the translation of few specific transcripts through alternative mechanisms of translation initiation[54] (Figure 3).

Control of overall mRNA translation by 4E-BP1

Upon activation 4E-BP1 interacts with eIF4E (see Introduction), thereby inhibiting mRNA translation initiation and reducing overall mRNA translation rates.[52] Earlier studies reported that recombinant 4E-BP1 induces inhibition of cap-dependent translation of capped luciferase or chloramphenicol acetyltransferase reporter transcripts.[9,55] Such effects are prevented by addition of recombinant eIF4E or when a 4E-BP1 mutant protein lacking the eIF4E-binding site is assayed.[9,55]

Selective inhibition of mRNA translation by 4E-BP1

Several studies revealed that, even though 4E-BP1-eIF4E impacts on overall mRNA translation activity,[52] the translation of a subset of transcripts is preferentially regulated by 4E-BP1-eIF4E at the initiation step.[53] A model for eIF4E-mediated selective translation emerged recently: because of its low expression rate, eIF4E is the least abundant translation initiation factor in most types of cells,

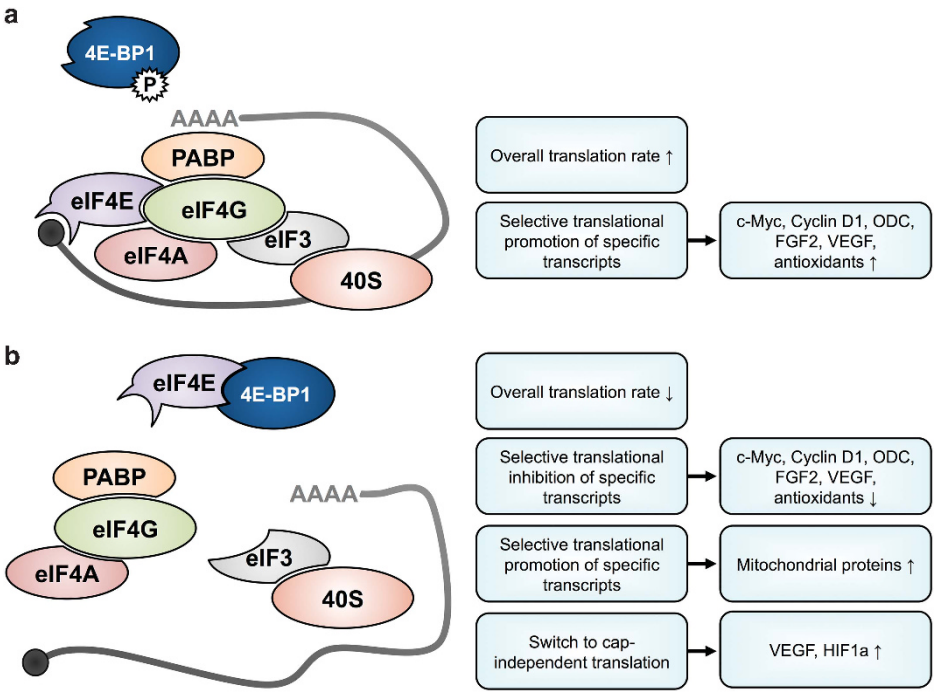

Figure 3. Schematic illustration of 4E-BP1 regulating eIF4F assembly and its impact on global and selective translation rates. (**a**) Phosphorylated 4E-BP1 is not able to bind eIF4E and to prohibit the eIF4F formation, resulting in an increase of overall translation rate and specific translation of pro-oncogenic transcripts. (**b**) Non-phosphorylated 4E-BP1 binds eIF4E, prohibits eIF4F formation and thus leads to a decrease in overall translation rate and a decrease in specific translation of pro-oncogenic transcripts. On the other hand, the eIF4E-binding also has pro-oncogenic properties by leading to selective translation of mitochondrial proteins and a switch from cap-dependent to cap-independent translation supporting selective mRNA translation of the pro-angiogenic factors HIF-1α and VEGF under hypoxia.

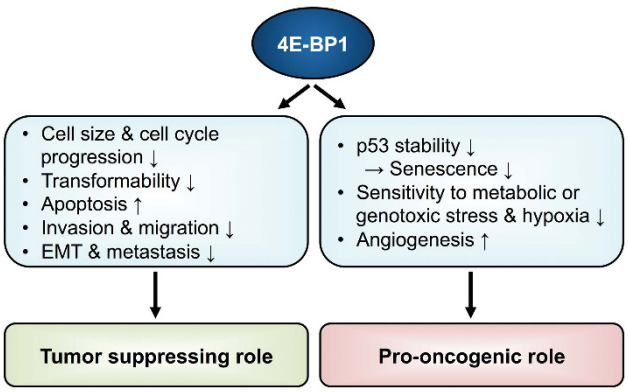

Figure 4. Overview on tumor suppressing and pro-oncogenic functions of 4E-BP1.

resulting in a competitive situation for mRNAs to become efficiently translated.[53] As a consequence, mRNAs with short and unstructured 5′UTR (as found in housekeeping genes), which are less dependent on the unwinding activity of the eIF4F complex, are efficiently translated but are largely unaffected by changes in eIF4E activity.[53] In contrast, the long, G/C rich and highly structured 5′UTR-containing mRNAs, which are typically found in mRNAs of proto-oncogenes, growth factors and angiogenesis factors (that is, weak mRNAs), are inefficiently translated when the active eIF4F complex is limiting.[53,56–66] However, when eIF4E levels, activity or availability are increased, the translation of such 'weak' transcripts is preferentially enhanced; these include mRNAs of c-Myc, Cyclin D1, ODC, FGF2 and vascular endothelial growth factor (VEGF).[53,56,67–71] Thus, 4E-BP1 will preferentially inhibit the translation of such pro-growth and pro-oncogenic transcripts by restraining eIF4E availability, which underlies its ability to block cell cycle progression[72–74] (see Physiological role of 4E-BP1 in cell cycle progression, cell growth and proliferation).

Recent studies employing a transcriptome-wide approach revealed a broader picture of the mRNA landscape translationally regulated by 4E-BP1-eIF4E. Using ribosome profiling, Thoreen *et al.*[75] highlighted that upon mTORC1 inhibition 4E-BP1 mediates selective translational repression of ribosomal protein and translation elongation factor mRNAs. Strikingly, these transcripts are characterized by the presence of a 5' terminal oligopyrimidine tract (5′TOP), a structural motif previously known to mediate selective translational repression following growth arrest.[76] The authors proposed that active 4E-BP1, by interacting with eIF4E, selectively prevents the binding of eIF4E to 5′TOP-containing transcripts.[75] Another study demonstrated that, in addition to transcripts of the translational apparatus, 4E-BP1 also represses translation of cell invasion and metastasis mRNAs.[77] Such regulation is critical for the impact of 4E-BP1 on tumor cell invasion (see 4E-BP1 as an inhibitor of tumorigenesis). Finally, very recent data indicate that the translation of transcripts encoding antioxidant proteins is selectively enhanced by eIF4E in transformed mouse embryonic fibroblasts (MEFs).[78] While the 5′UTRs of this subset of transcripts do not exhibit higher G/C content nor stronger secondary structure, they contain a cytosine-rich 15-nucleotide motif which may mediate the selective activity of eIF4E.[78]

Selective promotion of mRNA translation by 4E-BP1

Unexpectedly, it was reported that 4E-BP1 can positively regulate the translation of a subset of mRNAs.[54,79] In *Drosophila*, d4E-BP (the *Drosophila* 4E-BP1 ortholog) was shown to stimulate the translation of some transcripts encoding respiratory chain complex proteins and mitochondrial ribosomal proteins in

response to dietary restriction.[79] Indeed, overexpression of an active d4E-BP mutant leads to increased translation of reporter constructs containing the 5'UTR of some of these mRNAs.[79] The underlying mechanism has yet to be characterized, but interestingly these 5'UTRs are characterized by their short size and weak secondary structure.[79] Such d4E-BP selective translational control is critical to increase mitochondrial activity in response to dietary restriction, which supports lifespan extension of *Drosophila*.[79] In addition, another study demonstrated that 4E-BP1 may increase the translation of the pro-angiogenesis factors HIF-1α and VEGF under hypoxia through a cap-independent mechanism.[54] Overexpression of 4E-BP1, while restraining cap-dependent translational activity under hypoxia, promoted the translation of reporters containing the HIF-1α and VEGF internal ribosome entry sites (IRES), which are sequences known to mediate cap-independent translation.[54] Such translational regulation has profound effects on tumor angiogenesis (see 4E-BP1 as a promoter of tumorigenesis).

PHYSIOLOGICAL ROLE OF 4E-BP1 IN CELL CYCLE PROGRESSION, CELL GROWTH AND PROLIFERATION

Cellular proliferation requires a proper coordination between cell growth (that is, an increase in cell size and mass) and cell cycle progression, even though these are both two separable processes.[72,74] This is critical to maintain the proper size of individual cells and organs while cells divide.[72–74] Consequently, the relative rates of cell growth and cell cycle progression define the size of the resulting cells.[72]

A large body of evidence supports that the mTORC1 pathway regulates both cell growth and cell cycle progression. As mTORC1 senses growth factor and nutrient levels, it is critically involved in coupling the rate of cell growth to cell cycle progression.[72] Indeed it was shown that rapamycin inhibits cell cycle progression in all cell cycle phases, wherein cells in G1 phase are affected the most.[74,80] Braun-Dullaeus *et al*. report that hyperphosphorylated 4E-BP1 is associated with increased levels of cyclin B1, D1, E and Cyclin dependent kinases 1, 2 (CDK1 and CDK2) among others and that this overexpression can be blocked by rapamycin, whereas mRNA levels stay unaltered.[81] Consistently, Cyclin E levels, which are critical for G1/S transition, are lower in dTOR null mutant *Drosophila* leading to cell cycle arrest, which can be prevented by ectopic expression of cyclin E.[82] Enhancement of S6K1 activity or eIF4E overexpression increases cell size and accelerates S-phase entry,[73,74] while overexpression of a phosphorylation site-defective 4E-BP1 leads to a reduction of cell size[74] and of G1/S-phase transition rates.[73,74,82] Similarly, ectopic expression of overactive d4E-BP1 mutant in *Drosophila* causes smaller wing size by reduction of cell size and cell number.[83] Altogether these results indicate that mTORC1 signaling and 4E-BP1 play a central role in both cell growth and cell cycle progression.

However, this model proposing that both 4E-BP1 and S6Ks can indistinctly control both cell size and cell cycle progression downstream of mTORC1 has been challenged.[84] Indeed, Dowling *et al*. establish that cell cycle progression and cell size are separately regulated in higher eukaryotes by 4E-BP1 and S6Ks, respectively.[84] This study showed that depletion of Raptor by short-hairpin RNA (shRNA) impairs G1/S progression in wild-type MEFs, whereas this effect was not seen in 4E-BP1/2 double-knockout mouse embryonic fibroblasts (MEFs). In contrast, depletion of Raptor, serum deprivation or addition of an active-site TOR inhibitor (asTORi) decreases cell size to the same extent in both wild-type and 4E-BP1/2 double-knockout cells, indicating a role for 4E-BP1 in cell cycle regulation, but not in cell growth regulation. However, S6Ks were shown to have an impact on cell growth, but not on cell cycle progression.[84] Additionally, Lynch *et al*. reported that an overactive 4E-BP1 mutant can block cell proliferation, without influencing cell size.[85] Mechanistically, it was proposed that 4E-BP1 selectively inhibits the translation of transcripts required for cell proliferation such as those encoding ODC, cyclin D3 and VEGF[84] (see Selective inhibition of mRNA translation by 4E-BP1). Based on these results it is tempting to speculate that 4E-BPs regulate cell cycle progression but not cell growth in higher eukaryotes, whereas S6Ks play a crucial role in cell growth, but not in cell cycle progression.

THE ROLE OF 4E-BP1 IN PATHOPHYSIOLOGY OF CANCER

The mTORC1 pathway and its downstream effectors have a pivotal role in cell growth and cell cycle progression.[3,72–74] It is therefore expected, that the mTORC1 pathway, including 4E-BP1, is highly relevant in the pathophysiology of cancer, which is consistent with its frequent deregulation observed in various malignancies[86–137] (Table 1).

Role of eIF4E in cancer

Most oncogenic and tumor suppressing upstream effectors of 4E-BP1 were reviewed extensively[23–25,53,138–147] (Table 2). Thus, in order to understand the role of 4E-BP1 in cancer, it is necessary to characterize its downstream target eIF4E here, especially as eIF4E may act as an oncogene.[53] Indeed, overexpression of eIF4E stimulates cell proliferation and is sufficient to transform embryonic fibroblasts.[148–150] Conversely, genetic targeting of eIF4E abolishes Ras-mediated transformation of embryonic fibroblasts *in vitro* and *in vivo,*[151,152] whereas the overexpression of eIF4E in CREF fibroblasts induces the formation of both spontaneous and experimental metastases and leads to quicker metastatic spread when re-injecting eIF4E high expressing cells into nude mice.[65,66] Furthermore, eIF4E is able to enhance the selective synthesis of known oncogenes and cancer-promoting factors, such as c-Myc, Cyclin D1, ODC, FGF2 and VEGF, MMP9 and Heparanase.[53,145] Finally, eIF4E can prevent abnormal accumulation of reactive oxygen species by mediating the selective synthesis of antioxidant proteins to support cellular transformation.[78] These results indicate a crucial role for eIF4E in oncogenesis, angiogenesis and metastatic spread.

Consistently, eIF4E was shown to be overexpressed in many solid tumors and cancer cell lines (Table 1), such as colorectal, breast, bladder, lung, prostate, gastric, head and neck carcinomas, lymphomas and neuroblastomas.[53,153–162] A role for eIF4E as a prognostic marker has been suggested for certain cancers.[163] Increased expression of eIF4E is for example correlated with poorer clinical outcome and decreased survival in breast, head and neck, colorectal, lung, prostate, bladder, skin and cervical carcinomas, as well as in lymphomas.[1,53,164,165] Furthermore, eIF4E is associated with increased malignancy in meningiomas, glioblastomas, astrocytomas,[1,166] as well as decreased survival rates in advanced prostate cancer[1,128] and locally advanced esophageal cancer.[1,106,128]

4E-BP1: inhibitor or promoter of tumorigenesis?

In numerous cancer entities an overactivation of mTORC1 is reported, leading to enhanced 4E-BP1 phosphorylation/inactivation (Table 1). Such inactivation is expected to prevent 4E-BP1-mediated inhibition of the oncogenic eIF4E (see Role of eIF4E in cancer). However, several authors also report a role for non-phosphorylated and hence active 4E-BP1 in facilitating tumorigenesis, especially under conditions of cellular stress in the tumor's microenvironment (see 4E-BP1 as a promoter of tumorigenesis). It is therefore tempting to speculate that high levels of phosphorylated, inactive 4E-BP1 in a highly vascularized tumor promote tumor progression due to less translational inhibition of pro-oncogenic eIF4E-sensitive transcripts. In contrast, in the poorly vascularized center of a fast growing tumor, the cells adapt to starvation by not inactivating 4E-BP1, which may

selectively promote the translation of mRNAs that support survival of tumor cells under starvation (Figure 3). Figure 4 gives an overview on the tumor suppressing and pro-oncogenic functions of 4E-BP1.

4E-BP1 as an inhibitor of tumorigenesis. Overexpression of a constitutively active 4E-BP1 mutant is able to decrease cell size, to inhibit cell cycle progression (decrease of G1-progression rates), to suppress tumorigenicity and to mimick rapamycin treatment.[73,74,85,167] Furthermore, 4E-BP1 was shown to inhibit oncogene-mediated transformation of rat embryonic fibroblasts *in vitro* and *in vivo* by inhibiting cell proliferation and inducing apoptosis.[21,69,85] In prostate cancer cells, overexpression of a constitutively active 4E-BP1 mutant significantly decreases prostate cancer cell invasion without affecting cell cycle progression.[77] Conversely, silencing 4E-BP1 causes colon cancer epithelial cells to undergo epithelial-mesenchymal transition (EMT) and promotes cell migratory, invasive capabilities and metastasis.[168] In addition, Petroulakis *et al*.[169] demonstrated that 4E-BP1/2 double-knockout mice crossed with p53 knockout mice exhibit significantly shorter tumor-free survival than p53 knockout mice, indicating a tumor suppressor-like role for 4E-BP1. However, 4E-BP1 cannot be considered a genuine tumor suppressor on its own, as it was reported that 4E-BP1 knockout mice show no evidence of tumor development.[170]

The activation status of 4E-BP1 has been analyzed in numerous human tumor samples. In most cases, this was performed by assessing the levels of phospho-4E-BP1 (p-4E-BP1), whereas total 4E-BP1 levels were mostly not assessed. However, the mere anaylsis of p-4E-BP1 is not sufficient to determine whether 4E-BP1 is inactive. This requires determining the ratio between p-4E-BP1 and total 4E-BP1 levels. Given that data for total 4E-BP1 levels are lacking in most tumor samples analyzed for p-4E-BP1 levels, we argue that no definitive conclusion can be drawn on the activation status of 4E-BP1 in these samples. In addition, the ratio between eIF4E and 4E-BP1 levels is a determinant for 4E-BP1 activity as previously reported.[171] Therefore, further studies are warranted to determine p-4E-BP1:4E-BP1 ratio and eIF4E:4E-BP1 ratio in human tumor samples. It remains that overexpression of p-4E-BP1 is reported in a number of tumor entities, such as colorectal,[102] breast,[96,97] lung[115–117] and prostate carcinoma,[128] as well as leukemia[86,87] (Table 1). The level of p-4E-BP1 positively correlates with different outcome variables, such as poor prognosis, relapse, poor differentiation, tumor size, metastasis and tumor progression (Table 1). An increased eIF4E:4E-BP1 ratio is for example associated with higher risk for relapse in head and neck squamous cell carcinoma.[113]

4E-BP1 as a promoter of tumorigenesis. Paradoxically, Petroulakis *et al*. showed that 4E-BP1/2 double-knockout embryonic fibroblasts expressing p53 are resistant to Ras-mediated transformation and undergo cellular senescence instead. The authors propose a mechanism by which loss of 4E-BP1/2 enhances translation of Gas2 mRNA, encoding a p53 stabilizing protein, thereby leading to p53 accumulation and induction of cellular senescence.[169] In glioblastoma cells, the absence of 4E-BP1 renders tumors more sensitive to metabolic and genotoxic stresses. Indeed, 4E-BP1 knocked down tumors exhibit increased sensitivity to hypoxia-induced cell death *in vitro* and to radiation *in vivo* due to a decrease in the viable fraction of radioresistant hypoxic cells.[172] 4E-BP1 can further contribute to development of aggressive breast carcinoma by supporting tumor angiogenesis.[54] Advanced breast carcinomas were shown to overexpress 4E-BP1, leading to a hypoxia-induced switch from cap-dependent to cap-independent translation supporting the selective mRNA translation of pro-angiogenic factors HIF-1α and VEGF under hypoxia.[54] Consequently, knockdown of 4E-BP1 hampers growth of breast cancer xenografts as a result of reduced tumor vascular density.[54] Several studies reporting on 4E-BP1 levels in cancer show a correlation between 4E-BP1 levels and different variables indicating poor outcome.[93,103,107,109,131] High 4E-BP1 mRNA expression levels are reported to be an independent prognostic factor for poor outcome in breast cancer.[99,98]

CONCLUSION AND PERSPECTIVES

4E-BP1 is a central integration node of several signaling pathways sensing growth factor stimulation, nutrients or oxygen level. Thereby, 4E-BP1 mediates translational adaptation of tumor cells growing in variable microenvironments by regulating overall and selective mRNA translation. As an inhibitor of the pro-oncogenic eIF4E, 4E-BP1 is mainly described as a tumor suppressor. In contrast, 4E-BP1 may also promote tumor progression by selective mRNA translation, in particular under conditions of cellular stress, concluding that 4E-BP1 contains a dual role in tumor progression. Consistently, dysregulations in 4E-BP1's phosphorylation status or expression rate were described for many tumor entities and contain a prognostic value, although in future studies the levels of 4E-BP1, p-4E-BP1 and eIF4E have to be determined altogether, as the biological function of either protein depends on the expression and activation of the others. Nevertheless, 4E-BP1 not only has clinical relevance as a prognostic and/or predictive biomarker but also as a potential pharmacological target for a more specific and less toxic future anti-cancer therapy.

ABBREVIATIONS

(p-)4E-BP1, (Phosphorylated) Eukaryotic initiation factor 4E-binding protein 1; AMP, Adenosine monophosphate; AMPK, AMP-activated protein kinase; asTORi, Active-site TOR inhibitor; ATM, Ataxia telangiectasia mutated; ADP, Adenosine diphosphate; AMP, Adenosine monophosphate; ATP, Adenosine triphosphate; BNIP, BCL2/adenovirus E1B 19-kDa protein-interacting protein; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CDK1,2, Cyclin dependent kinases 1, 2; CREF, Cloned rat embryo fibroblast; DNA, Desoxyribonucleic acid; EGFR, epidermal growth factor receptor; eIF3, Eukaryotic initiation factor 3; eIF4E,A,G, F, Eukaryotic initiation factor 4 E, A, G, F; EMT, Epithelial-mesenchymal transition; ERK, Extracellular signal-regulated kinase; FGF2, Fibroblast growth factor 2; Gas2, Growth arrest-specific protein 2; GSK3, Glycogen synthase kinase 3; GTP, Guanosine triphosphate; HIF-1α, Hypoxia-inducable factor 1-α; IKKβ, Inhibitor of kappa light polypeptide gene enhancer in B-cells kinase beta; IRES, Internal ribosome entry sites; LKB1, Liver kinase B 1; MAPK, Mitogen-activated protein kinase; MEF, Mouse embryonic fibroblast; MMP9, Matrix metalloproteinase 9; (m)RNA, (Messenger) ribonucleic acid; mTOR, Mammalian target of rapamycin; mTORC1, 2, mTOR complex 1, 2; ODC, Ornithine decarboxylase; PABP, Poly-A-binding protein; PA, Phosphatidic acid; PDGFRB, platelet-derived growth factor receptor, beta polypeptide; PI3K, Phosphatidyl-inositide-3 kinase; PML, Promyelocytic leukemia; PTEN, Phosphatase and tensin homolog; Raptor, Regulatory-associated protein of mTOR; REDD1, Regulated in development and DNA damage response 1; Rheb, Ras homolog enriched in brain; RSK1, Ribosomal S6 kinase 1; S6K1,2, Ribosomal protein S6 kinase 1, 2; shRNA, Short-hairpin RNA; TNFα, Tumor necrosis factor α; TNM, tumor-nodes-metastasis classification; TOP, Terminal oligopyrimidine tract; TSC, Tuberous sclerosis complex; UTR, Untranslated region; v-ATPase, Vacuolar H +- ATPase; VEGF, Vascular endothelial growth factor.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

JM, BR, TK, GL and TGPG conceived and wrote this paper. All authors contributed in literature survey as well as in drafting of the figures and the tables.

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