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THE P53-INDUCED WIG-1 PROTEIN: IDENTIFICATION OF mRNA TARGETS AND ROLE AS A SURVIVAL FACTOR IN DEVELOPMENT AND CANCER

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THE P53-INDUCED WIG-1 PROTEIN: IDENTIFICATION OF mRNA TARGETS AND ROLE AS A SURVIVAL FACTOR IN DEVELOPMENT AND CANCER

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To my beloved family

前言

本论文及其包含的工作是探讨癌症形成机理的基础研究。癌症已逐渐成为威胁人类生存的头号杀手。那么为什么人会得癌症?

人身体结构和功能的基本单位是细胞，一个成年人由约 60 万亿个细胞组成。在人的一生中，大部分细胞都在严格的调控下进行分裂增殖，从而每天产生数百万个新细胞来替代受损或老化的细胞。但是，在细胞分裂增殖这一过程中难免会有错误发生。多数时候这些错误会被人体自身的免疫系统自动纠正、修复或者清除。然而，少数没被纠正的错误则会不断累积，当这些错误累积到一定阶段，某个携带错误信息的细胞则会发展成癌细胞，癌细胞进一步分裂出更多的子癌细胞，从而形成癌症。癌细胞的共同特点是不受细胞外信号的调控，会无节制的生长而不会死亡，同时逃脱免疫系统的监管，并且有能力转移到身体其他组织。

癌症的发生并非一日之寒，它是长时间内因和外因作用下共同诱发的。人体内有大约 20,500 个蛋白编码基因，其中两类和癌症形成发展息息相关的基因被称为“原癌基因”和“抑癌基因”。在正常细胞中，原癌基因与抑癌基因共同维持细胞的正常增殖活动。但这两类基因一旦发生编码错误，如突变、片段缺失和过多复制等，就成为驱动癌细胞形成的内因。癌细胞形成的外因则包括各种外界刺激，比如放射线、尼古丁、多环芳烃类化合物、病毒等。

抑癌基因 p53，在细胞里调控着许多关键的信号通路。一旦细胞受到外界刺激，p53 会被激活，触发细胞凋亡或细胞周期停滞等信号通路的开放，从而抑制细胞的癌变。p53 突变则会导致其丧失对细胞的保护作用。目前已知的癌症中 p53 基因的突变率高达 95%。在对 p53 所调控关键信号通路的研究让我们更清楚地了解癌变的同时，这些信号通路中的重要因子也日益成为抗癌药物筛选和癌症治疗中的关键靶点。

本论文研究重点是 p53 转录调控的一个下游基因 Wig-1。Wig-1 是在 Klas Wiman 教授领导的实验组被首先发现并命名的。Wig-1 蛋白是对 mRNA 起调控作用的锌指蛋白，它通过附着在 mRNA 的一段特殊的区域从而对 mRNA 进行调控。已经被证实的 Wig-1 的作用对象有 p53、癌基因 Myc、调控细胞周期因子 p21 等。

Paper I 研究证实了 Wig-1 的其他调控对象，包括促细胞凋亡基因 FAS 和调控细胞周期因子 14-3-3σ 。paper II 发现了 Wig-1 蛋白在宫颈癌病人肿瘤样本中的表达强度和宫颈癌病人生存率的相关性，揭示了 Wig-1 基因表达在宫颈癌诊断及预后中起到的作用。Paper III 探讨了 Wig-1 在胚胎发育中的重要作用，发现 Wig-1 基因的敲除会导致小鼠胚胎期的死亡。Paper IV 进一步探索了 Wig-1 更多调控对象在结构特征上的相似之处。

此论文是本人四年多博士工作的成果，全部工作均是在卡罗琳斯卡医学院 (Karolinska Institutet) 肿瘤中心完成。卡罗琳斯卡医学院建立于 1810 年，位于瑞典斯德哥尔摩，是全球高等教育中最大的一所医学大学。其中的诺贝尔委员会，包括我的导师 Klas Wiman 在内，专门负责评审及颁发诺贝尔生理医学奖。

ABSTRACT

The tumor suppressor p53 is activated in response to a variety of stress conditions. Upon activation, p53 can trigger apoptosis or cell cycle arrest, or regulate metabolism and other cellular processes by transactivation of its targets genes. Wig-1 (also named ZMAT3) is a p53 target gene, and both Wig-1 mRNA and protein levels increase upon p53 activation. Wig-1 is a zinc finger protein that binds to double strand RNA. It is an AU-rich element (ARE) binding proteins (ARE-BPs) and acts as a regulator of mRNA stability via direct binding to AREs. The Wig-1 gene is localized to the long arm of chromosome 3(3q26), a region that is frequently amplified in cancer.

In this thesis, I aimed to identify new Wig-1 mRNA targets and investigate the biological implications of the regulation of selected targets. I also explored Wig-1 protein expression in tumors as well as its clinical relevance, and determined the role of Wig-1 in mouse development.

In paper I, we performed a microarray analysis of HCT116 colon cancer cells with or without Wig-1 knockdown and identified Wig-1 regulated mRNAs. We also discovered that Wig-1 promotes cell cycle arrest rather than cell death upon cellular stress through regulation of p53 targets FAS and 14-3-3σ. Wig-1 regulates FAS mRNA negatively through binding to 3'UTR AREs in FAS mRNA.

In paper II, we studied Wig-1 expression in cervical carcinoma samples and found that the Wig-1 protein expression pattern in tumors is associated with patient survival. Patients with moderate nuclear Wig-1 staining and positive cytoplasmic Wig-1 staining in their tumors show better survival than patients with high nuclear Wig-1 staining and negative cytoplasmic Wig-1 staining.

In paper III, we showed that Wig-1 null mice embryos die before the blastocyst stage. We also found that Wig-1 knockdown in mouse embryonic stem cells (mESCs) leads to a reduction in proliferation rate. Wig-1 binds to and regulates both c-Myc and N-Myc mRNA in mESCs. Since Myc has essential roles during embryonic development, we suggest that deficient regulation of Myc in absence of Wig-1 may explain the observed embryonic lethality.

In paper IV, we performed RNA-immunoprecipitation (RIP) followed by high-throughput RNA sequencing in HCT116 and Saos2 cells. We identified Wig-1 bound mRNAs and found a significant enrichment of mRNAs with AREs in their 3'UTRs as compared to unbound mRNAs.

In summary, this thesis greatly expands theWig-1 targets repertoire and further explores the role of Wig-1 as a survival factor in cell growth and early embryonic development. Our findings also suggest that Wig-1 may serve as a molecular biomarker together with other conventional clinical markers for cervical cancer prognosis.

LIST OF PUBLICATIONS

I. Cinzia Bersani**, Li-Di Xu,** Anna Vilborg, Weng-Onn Liu, Klas G. Wiman.
Wig-1 regulates cell cycle arrest and cell death through the p53 targets FAS and 14-3-3σ
Oncogene. 2014 Aug 28; 33(35): 4407-17

II. **Li-Di Xu**, Susanne Muller, Srinivasan R.Thoppe, Fredrik Hellborg, Lena Kanter, Mikael Lerner, Biying Zheng, Svetlana Bajalica Lagercrantz, Dan Grander, Keng Ling Wallin, Klas G. Wiman, Catharina Larsson, Sonia Andersson.
Expression of the p53 target Wig-1 is associated with HPV status and patient survival in cervical carcinoma
PLoS ONE. 2014 Nov 7; 9 (11): e111125.

III. **Li-Di Xu,** Anna Vilborg, Sophia Ceder, Cinzia Bersani, Björn Rozell, Klas G. Wiman, Margareta T. Wilhelm.
Complete lack of Wig-1 leads to embryonic lethality before the blastocyst stage
Manuscript

IV. Cinzia Bersani, Mikael Huss, Stefania Giacomello, **Li-Di Xu,** Julie Bianchi, Sofi Eriksson, Fredrik Jerhammar, Anna Vilborg, Andrey Alexeyenko, Weng-Onn Liu, Klas G. Wiman
Genome-wide identification of Wig-1 mRNA targets by RIP-Seq analysis
Oncotarget, under revision

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LIST OF ABBREVIATIONS

ADCA	adenocarcinoma
AGO	argonaute
AMD	AU-rich element mediated decay
ARE	adenylate-uridylate rich element
AUF1	AU-rich element binding factor-1
BRF1	butyrate-related factor-1
CAV1	caveolin 1
CHIP	chromatin immunoprecipitation
CNOT6	CCR4-NOT transcription complex, subunit 6
Co-IP	co-immunoprecipitation
COX-2	cyclooxygenase-2
Dcp2	decapping enzyme2
DICER	double-stranded RNA-specific endoribonuclease 1
DNA	deoxyribonucleic acid
dsRNA	double-stranded RNA
eIF4E	eukaryotic Translation Initiation Factor 4E
ELAVL1	embryonic lethal, abnormal vision, homolog-like 1
ESC	embryonic stem cell
FAS	FAS cell surface death receptor
GU-rich	guanylate-uridylate-rich
HIF1A	hypoxia inducible factor 1, alpha subunit
HnRNPD	heterogeneous nuclear ribonucleoprotein D
HPV	human papillomavirus
HR	high risk
HuD	human antigen D
HuR	human antigen R
IHC	immunohistochemistry
IVF	in vitro fertilization
JAZ	just another zinc finger protein
KSRP	KH-domain-splicing regulatory protein
LCLs	lymphoblastoid cells

MAX	MYC associated factor X
miRNA	microRNA
mRNA	messenger RNA
N-Myc	v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog
PAG608	p53-activated gene 608
Pan2/Pan3	poly(A)-binding protein (PAB)-specific ribonuclease 2/3
PARN	poly(A)-specific ribonuclease
PCR	polymerase chain reaction
PIG3	p53-induced gene 3
PIK3CA	phosphatidyl inositol-3-kinase, catalytic subunit alpha
PTM	post-translational modification
PUMA	p53-upregulated modulator of apoptosis
RB	retinoblastoma protein
RIP	RNA-immunoprecipitation
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
RNA-FISH	RNA-fluorescence in situ hybridization
ROS	reactive oxygen species
SCC	squamous cell carcinoma
SCLC	small cell lung cancer
SG	stress granules
shRNA	small hairpin RNA
siRNA	small interfering RNA
SOX2	SRY (sex determining region Y)-box2
TF	transcription factor
TNF-α	tumor necrosis factor alpha
TIA-1	T-cell intracellular antigen 1
TIAR	TIA-1 related protein
TP53	tumor protein 53
TTP	Tristetraprolin
UTR	tupstream open reading frame
Wig-1	wild-type p53 induced gene 1

Xnr1	5’ to 3’ exoribonuclease 1
ZMAT3	zinc finger, matrin-type 3
ZF	zinc finger

1 THESIS SUMMARY

1.1 INTRODUCTION

1.1.1 Cancer

Cancer is responsible for one in seven deaths worldwide[1]. Cancer develops as a result of deficient control of cell growth and cell survival. At later stages, tumors may gain the ability to invade other tissues and form distant metastases. Cancer can arise in almost any tissue in the human body and is classified according to the cell type in which is originates (Figure 1).

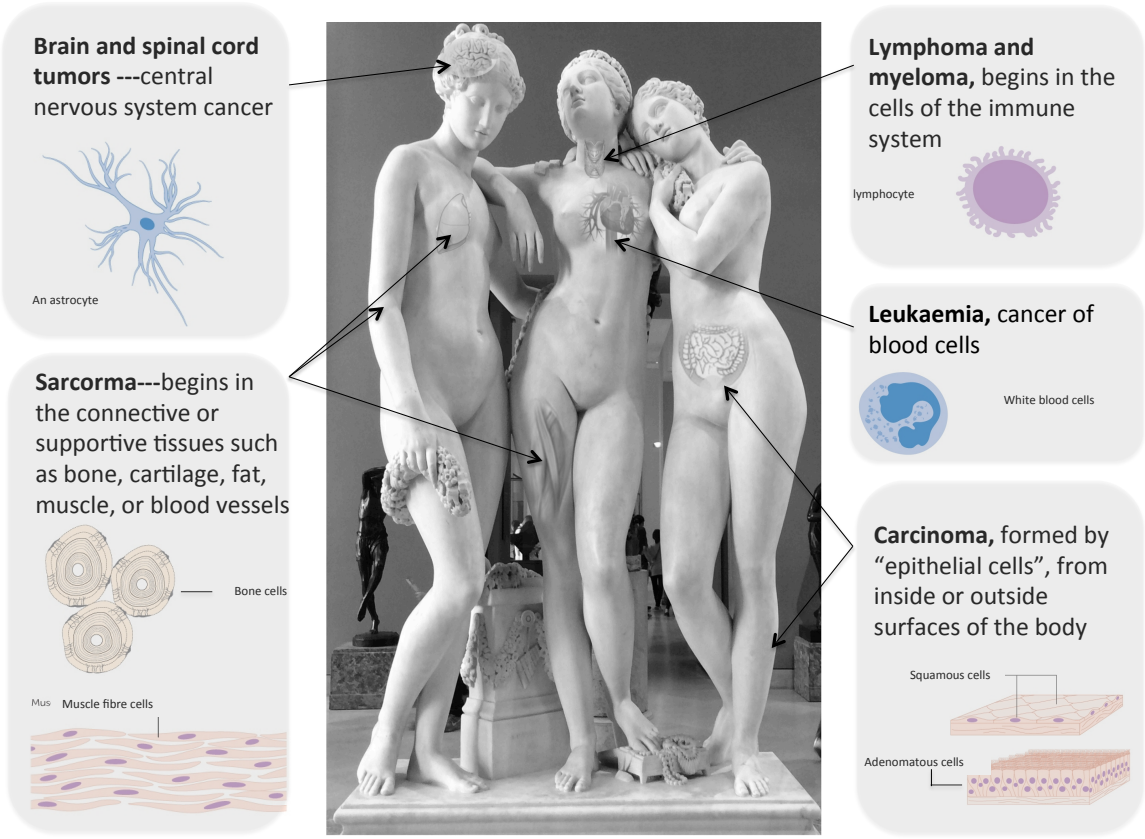


Figure 1. Five major cancer types. (main source: http://www.cancerresearchuk.org. Photo of the three Graces marble statue was taken in the Louvre Museum in Paris.

All of these different types of cancer share 10 common features defined by Hanahan and Weinberg as the Hallmarks of cancer. (1) Cancer cells divide in an uncontrolled manner -

- ”sustain proliferation by their own”; (2) they are “blind” and “deaf”, as they ignore commands from the extracellular environment; (3) they resist cell death and evade growth suppressions; (4) they stimulate the growth of blood vessels to get nutrients; (5) they are “greedy”-- since they ignore cellular contact inhibition and become invasive and able to metastasize; (6) they are immortalized with unlimited replicative potential; (7) they alter the normal energy metabolism; (8) they are genomically instable and have accelerated mutation rates; (9) they avoid immune destruction; (10) they induce inflammatory responses, thus supplying bioactive molecules that facilitate the other hallmarks [2,3].

Underlying these Hallmarks are genomic alterations, such as mutations, deletions and amplifications, in two major groups of genes: proto-oncogenes and tumor suppressor genes[4–7]. Alterations in proto-oncogenes convert them into oncogenes and drive uncontrolled cell proliferation. For example, the c-Myc oncogene is activated by chromosomal translocation in Burkitt lymphoma[8,9], and the N-Myc oncogene is amplified in for instance neuroblastoma [10,11]. Alterations in tumor suppressor genes, on the other hand, abolish their tumor suppressor function. For example, the tumor suppressor gene *TP53* is the most frequently mutated gene in cancer[5]. Mutant p53 has lost its ability to induce cell cycle arrest or cell death by apoptosis, and/or regulate metabolism and other cellular processes upon DNA damage or oncogenic stress. This allows tumor growth. For this reason, the p53 protein has been named the “guardian of the genome” [12].

1.1.2 The p53 tumor suppressor

1.1.2.1 Discovery of p53

Since its discovered in 1979[13,14], the p53 has been the focus of intense research worldwide. The mutation frequency of p53 in cancer ranges from 2% to 95% depending on the cancer type[5] (www.cbioportal.org). Inherited germline p53 mutations are the underlying genetic defect in the Li-Fraumeni syndrome, a familial syndrome comprising multiple cancers such as osteosarcomas, breast cancer, soft tissue sarcoma and leukemia[15,16], developing at an early age. Moreover, p53 can acquire “gain-of-function

mutations", that is, mutations that confer new or enhanced activity, promoting tumor proliferation rather than inhibiting cell growth[17,18]. The tumor suppressive function of p53 is also supported by studies in mice[19,20]. Complete lack of p53 in mice leads to spontaneous development of a variety of neoplasms by 6 months of age and death before 9 months of age[19].

1.1.2.2 p53 and cell fate decision

The p53 protein is a key transcriptional factor that is activated in response to cellular stress, including DNA damage, oxidative stress or oncogenic stress, thus inducing many critical cellular functions (reviewed in[21–23]). It has been suggested that low levels of stress induce p53-mediated cell cycle arrest, DNA repair, stem cell maintenance, and antioxidant response and regulates fertility, metabolism, while high stress levels trigger apoptosis, senescence, stem cell erosion and/or pro-oxidant activity ([24],reviewed in[22,25–28]). This is summarized in Figure 2.

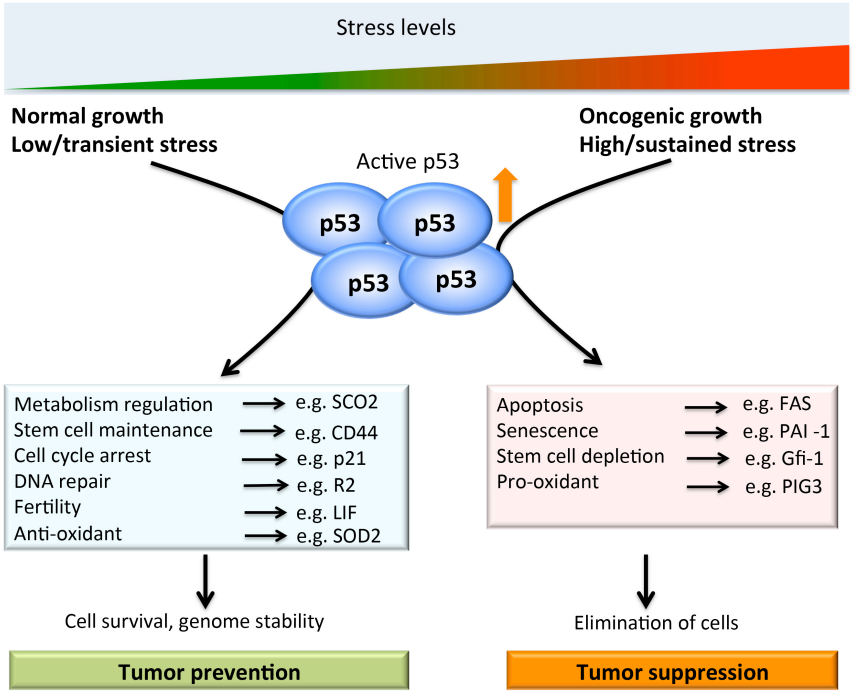


Figure 2. Schematic representation of p53 responses upon different stress levels, and its respective regulated pathways.

A growing list of genes that are transactivated by p53 have been identified and their contributions to different p53 regulated pathways is well established in many cases. For

example, p53 induces cell cycle arrest mainly through its targets p21 (other names WAF1/CIP1/CDKN1A)[29] and 14-3-3σ[30], and triggers apoptosis through pro-apoptotic genes such as PUMA[31], NOXA[32] and FAS[33]. p21 is also known to be a key regulator in senescence[34,35].

If the task of researches in biology is to reduce the complicated to the simple and illustrate the beauty of simplicity in nature, the study of p53 has made this task a lot more challenging. Although p53-mediated cell-cycle arrest, senescence, and apoptosis are believed to be prime barriers against tumor growth, emerging evidence suggests that this is not the full picture. Li et al has shown that while $p53^{3KR/3KR}$ cells carrying the p53 acetylation mutant K117R/K161R/K162R which fails to activate p21 and PUMA and cannot enter cell cycle arrest, apoptosis and senescence; mice with the $p53^{3KR/3KR}$ mutation do not develop early onset of thymic lymphomas[36]. In another study by Valente et al., thymocytes derived from p21-/- Puma-/- Noxa-/- triple knockout mice were unable to undergo p53-mediated cell cycle arrest, apoptosis, and senescence. Nevertheless, unlike p53-/- mice, none of these mice developed a tumor (or other diseases) within the 500 days observation period[37.]

The above findings suggest that p53-induced cell cycle arrest, senescence and apoptosis are dispensable for p53-mediated tumor suppression and raise the possibility that other "unconventional" p53-mediated cellular processes, e.g. metabolism[38] and DNA damage repair[37], may be more critical to the tumor suppressor or activity of p53. Preliminary data have suggested that Wig-1 is a key mediator of p53's tumor suppressor function. An shRNA library screen led to the identification of Wig-1 as a candidate gene whose inactivation induces lymphomas in 50% of mice transplanted with p21-/- Puma-/- double knock out hematopoietic stem/progenitor cells (HSPCs) within 350 days (A. Strasser, Melbourne, personal communication).

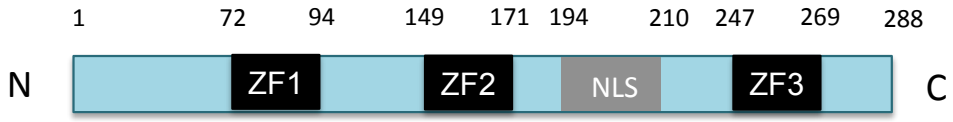
1.1.3 The Wig-1 gene and protein

WIG-1 (WT p53-induced gene 1, also known as *ZMAT3* or *PAG608*) is a p53 target gene that has previously been identified in our lab[39]. By using a PCR-based differential display technique, two *WIG-1* transcripts were found to be upregulated by wtp53 expressed as a

temperature-sensitive mutant p53 in mouse T lymphoma cells[39]. *WIG-1* (*PAG608*) was characterized independently in rat by another research group at almost the same time[40].

The human *WIG-1* was identified subsequently and localized to 3q26.32[41]. Due to alternative splice site usage, human Wig-1 is expressed as two main transcript variants corresponding to two isoforms with 288 and 289 amino acids residues, respectively. On the other hand, mouse Wig-1 is expressed as one isoform containing 290 amino acids.

The Wig-1 protein contains three widely spaced zinc finger (ZF) motifs of the Cys2-His2 type (residues 72-94, 149-171 and 247-269) and a nuclear localization signal (residues 194-210). The Wig-1 zinc fingers are special in two aspects: (1) the inter-histidine distance within the zinc fingers are five amino acids compared to the usual three to four amino acids; (2) the long linkers between the zinc fingers are 56-75 amino acids instead of six to eight as in most other zinc finger proteins. The first two ZFs show strong homology to each other while the third ZF motif does not, except that the cysteins and histidines are conserved. The Wig-1 proteins are highly conserved at the protein level with 100% amino acid sequence identity between human and primates such as chimpanzee, gorilla, orangutan and gibbon, and 87% amino acid identity between human and mouse Wig-1. Moreover, the ZFs are almost completely conserved all the way from amoeba to human Wig-1 [42] (Figure 3).



MILLQHAVLPPPKQPSPSPPMSVATRSTGTLQLPPQKPFGQEASLPLAGEEELSKGGEQD
CALEELCKPLYCKLCNVTLNSAQQAQAHYQGKNHGKKLRNYYAANSCPPPARMSNVV
EPAATPVVPVPPQMGSFKPGGRVILATENDYCKLCDASFSSPAVAQAHYQGKNHAKRL
RLAEAQSNSFSESSELGQRRARKEGNEFKMMPNRRNMYTVQNNSGPYFNPRSRQRIPRD
LAMCVTPSGQFYCSMCNVGAGEEMEFRQHLESKQHKSKVSEQRYRNEMENLGYV

Figure 3. Human Wig-1 protein structure and its sequence (three zinc fingers and NLS are highlighted in the sequence).

Human Wig-1 is expressed as two proteins, one full-length Wig-1 species and one that lacks the 20 N-terminal amino acid residues, due to translation initiation from different ATGs. The full-length protein is initiated from the main ATG residing in a relatively weak context, while the smaller protein is initiated from a downstream in-frame ATG. Translation initiation at the downstream ATG is explained by the existence of an out of frame ATG situated 19bp upstream of the main ATG and a short open reading frame. The two Wig-1 species are visualized by Western blotting using a rabbit polyclonal antibody raised against the full-length protein[43] and another rabbit polyclonal antibody against the C-terminus.

The unusual zinc finger structure of Wig-1 is shared by a small group of double-stranded RNA (dsRNA) binding proteins[44], with Jaz as the most well known protein[45]. Indeed, we have found that Wig-1 binds to both long and short dsRNA[46]. The first and second zinc fingers are critical for binding.

The crystal structure of Wig-1 has not been determined, but ModBase[47], a database of comparative protein structure models, provides a predicted 3D structure model for a fragment of the Wig-1 protein (https://modbase.compbio.ucsf.edu).

1.1.4 Wig-1 is an AU-rich element binding protein

1.1.4.1 What is an AU-rich element?

Adenylate-uridylate-rich elements (AREs) within 3' untranslated regions (3'UTR) are signals for rapid degradation of mRNAs[48,49]. AREs are one of the most prevalent cis-acting elements site in 3'UTRs[48,50,51] that can interact with AU-rich element binding proteins (ARE-BPs). Approximately 17% of human mRNAs are estimated to contain one or several AREs motifs as reported by AREsite, a database for investigating AREs in vertebrate[52]. AREs containing mRNAs are a functionally diverse group of proteins including many oncoproteins (e.g. c-Fos[53], c-Myc[54]) and cytokines (e.g. tumor necrosis factor TNF-α[55], interleukin 6[56]). Most of them play essential roles in cell proliferation, differentiation and in the immune response (reviewed in[57]).

1.1.4.2 What is an AU-rich element binding protein?

ARE-BPs are proteins that bind to AREs and have roles in controlling posttranscriptional gene expression [58,59]. AREs in different mRNAs can be bound by more than one protein under different conditions, and one ARE-BP can bind to more than one ARE-containing mRNA. More than 20 ARE-BPs have been identified so far and they can be divided into three distinct groups (reviewed in Barreau et al. 2005): (1) mRNA destabilizing proteins, such as tristetraprolin (TTP) [60,61], butyrate-regulated factor-1 (BRF1/ZFP36L1)[61,62], and KH domain-splicing regulatory protein (KSRP)[63,64]; (2) mRNA-stabilizing proteins, such as embryonic lethal abnormal vision (ELAV)-like protein 1 (also named HuR)[65]; and (3) proteins promoting both mRNA degradation and stability such as ARE binding factor-1 (AUF1/hnRNP D)[66,67].

Most ARE-BPs cause destruction of their binding mRNAs through an ARE-dependent decay by recruiting the degradation machinery [68,69]. In addition, they can also regulate mRNA translation [57,70]. These two post-transcriptional regulation mechanisms of ARE-BPs will be discussed in the following sections.

Additionally, ARE-BPs are also involved in regulating mRNA localization, mRNA trafficking, miRNA maturation and several other aspects of RNA biology (reviewd in[71]).

As discussed above, ARE-containing mRNA are mostly oncogene and inflammatory mediators, and therefore it is not surprising that changes of ARE-BPs expression levels are associated with tumor growth[72–74]. ARE-BPs are also involved in promoting angiogenesis[75,76] and metastasis[77], both known hallmarks of cancer, indicating that ARE-BP expression levels may be useful prognostic and predictive markers in cancers and that ARE-BPs could also be potential targets for cancer treatment[78].

Furthermore, ARE-BPs have essential roles in stem cell proliferation and embryogenesis[79–81]. Brf1-deficient mutants mice develop abnormal placenta and die during the midgestation stage[79]. HuR null embryos show defects in limb, skeletal and spleen development and fail to survive beyond midgestation. This lethality is due to deregulation of several HuR target mRNAs[81]. These findings suggest that ARE-

containing mRNAs and their binding proteins are subject to fine-tuned regulation during development.

1.1.4.3 ARE-mediated decay (AMD) and ARE-BPs

ARE-mediated decay (AMD) promotes mRNA degradation mainly through two different mechanisms: exonuclease-mediated decay and endonuclease-mediated decay[82,83] (Figure 4). Exonuclease mediated decay begins with shortening of the poly(A) tail (deadenylation). This is mediated mainly through three groups of enzymes: (1) cytoplasmic deadenylase complexes which are poly(A)-binding protein (PAB)-specific ribonuclease 2 (PAN2) and PAN3[84]; (2) CCR4–NOT transcription complex subunit 6 (CNOT6), CNOT6L, CNOT7 and/or CNOT8[85]; and (3) the deadenylase poly(A) ribonuclease (PARN). It is then followed by removal of the 5' cap (decapping) through the Nudix domain proteins mRNA-decapping enzyme 2 (DCP2) or NUDT16 [86]. Then degradation of the mRNA body proceeds from both ends, with 5'-to-3' degradation by 5'-to-3' exoribonuclease 1 (XRN1) and/or 3'-to-5' degradation by exosomes (Figure 4A).

The endonuclease-mediated AMD is, on the other hand, initiated by endonuclease cleavage within the body of the mRNA, followed by degradation of the upstream cleavage product by the exosome and degradation of the downstream cleavage product by XRN1[87] (Figure 4B). Another group of endoribonucleases is represented by Argonaute (AGO), a component of the RNA-induced silencing complex (RISC). Association between AGO and miRNA, short RNA molecules (20-22nts) involved in post-transcriptional regulation of mRNA[88] has shown to mediate the AMD effect[89].

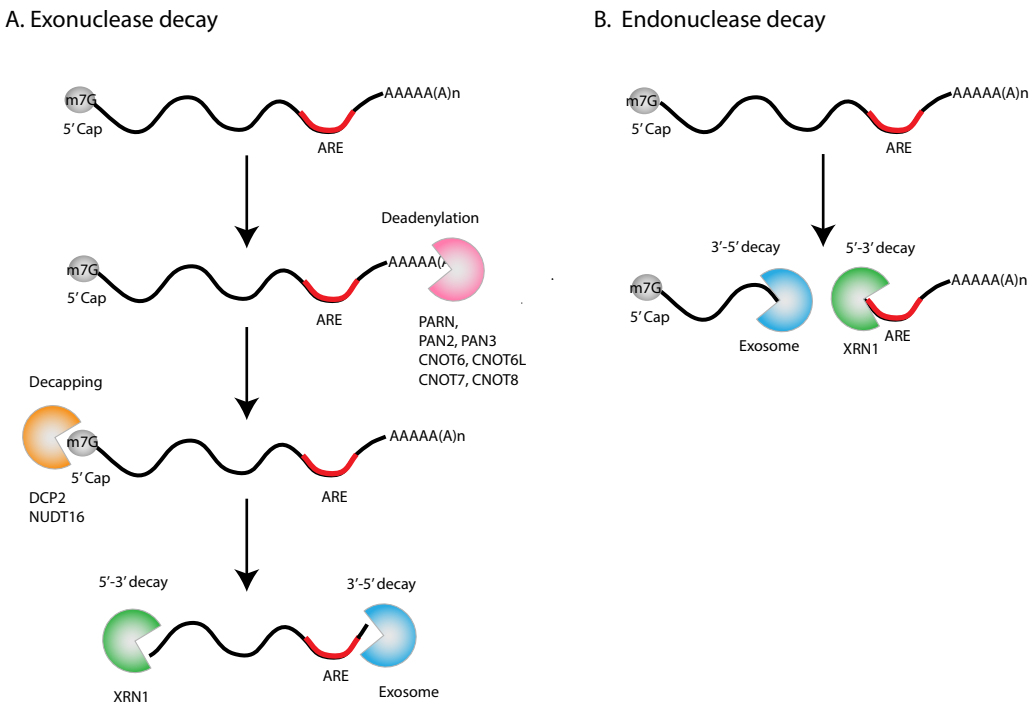


Figure 4. ARE-mediated decay (AMD) mechanisms. A. exonuclease decay pathway. B. endonuclease decay pathway.

ARE-BPs mainly exert the destabilizing effects on target mRNAs by recruiting these deadenylases or endoribonucleases to the mRNA, or stabilizing the mRNA through interference with each individual step of AMD[90,91]. miRNA can bind to ARE-BPs and enhance or inhibit the regulation functions of ARE-BPs[92–95].

1.1.4.4 Translational regulation by AREs and ARE-BPs

A contribution of ARE-BPs to mRNA translation control has also been reported. The ARE-BP named T-cell intracellular antigen 1 (TIA-1) has a translational repressive effect on the cytokine TNF-α [96] and the enzyme cyclooxygenase-2 (COX-2)[97]. HuR, for example, was also reported to act as both a positive and a negative translational regulator. Elevated HuR expression greatly enhanced translation of p53 mRNA with no effect on p53 mRNA abundance[98,99], while on the other hand, overexpression of HuR inhibited polysome recruitment of TNF-α and COX-2[100].

However, the exact mechanism for this translational regulation by ARE-BPs is not well understood. One possibility is that ARE-BPs regulate translation through recruitment of

ribosomes. This is supported by studies showing that the RNA-binding protein fragile-X-mental-retardation-related protein 1 (FXR1) interacts with AGO2, which then associated with an ARE in TNF-α mRNA. This complex is then recruited by FXR1 to ribosomes, thereby upregulating TNF-α mRNA translation[70]. A possible alternative mechanism involves stress granules (SGs), a type of cytoplasmic granule that can be induced upon stress[101]. ARE-BPs can recruit their target mRNAs to stress granules in response to a variety of stresses in an ARE-dependent manner[102,103]. mRNAs in SGs are subjected to storage, stabilization or decay largely depending on the duration and severity of the stress[104]. Once the stress is gone, SGs dissemble and release mRNA and its associated ARE-BPs that any then be available for translation reinitiation[103,105].

1.1.4.5 Wig-1 function

Previous studies have shown that Wig-1 is amplified and overexpressed in squamous cell carcinoma of the lung[106]. Ectopic overexpression of Wig-1 was shown to inhibit cell growth[41]. Additionally, overexpression PAG608, the rat homolog of Wig-1, promotes cell apoptosis[40]. Interestingly, both overexpression of exogenous Wig-1 and knockdown of endogenous Wig-1 can inhibit cell growth[107]. These studies indicate that Wig-1 levels are tightly regulated in the cell and that a well-balanced expression of Wig-1 is necessary for cell normal growth.

Wig-1 knockdown in mouse liver and brain by using antisense oligonucleotides caused changes in expression of several genes, a number of which were shown to be relevant for both nervous system function and cancer progression[108].

Our lab has demonstrated that Wig-1 is an ARE-BP that acts as a regulator of mRNA stability through direct binding to AREs in the 3'UTR of its target mRNAs. Wig-1 stabilizes p53 mRNA by binding to an ARE in the p53 3'UTR and preventing mRNA deadenylation[109]. By doing this, Wig-1 enhances p53 protein expression levels and potentiates the p53 stress response. Wig-1 was also shown to bind to a stem loop structure on the 3' UTR of the p53 target p21 mRNA and to recruit the miRNA-associated RISC complex to its target site, thereby promoting to miRNA-mediated p21 decay, which resulted in premature senescence[110].

1.1.5 Regulation of Wig-1

1.1.5.1 Transcriptional regulation

How is Wig-1 regulated? Both Wig-1 mRNA and protein are induced by DNA damaging agents such as gamma radiation and cisplatin in a p53 dependent manner[39,40]. Wig-1 is a bona fide p53 target gene as confirmed by several studies[111–113]. The mouse *WIG-1* promoter contains three putative p53-binding motifs. Two of them form a strong complex with p53 and contribute significantly to p53-dependent transactivation as demonstrated by electrophoretic mobility shift assays[114]. Human *WIG-1*, on the other hand, has a perfect consensus p53 response element in intron 1 (F. Hellborg et al., unpublished data).

Yet we have found that Wig-1 is expressed also in cells lacking p53, indicating that p53 is not the only transcriptional factor that regulates Wig-1 expression. Indeed, by using CSCAN[115], a web resource that includes a large collection of genome-wide ChIP-Seq experiments, we have identified a number of novel transcriptional factor (TFs) that could regulate Wig-1 expression in different cell lines (Table 1). This suggests that Wig-1 is regulated by different TFs under different conditions.

Table 1. List of transcriptional factors binding to either human or mouse *WIG-1* promoters (source: CSCAN)

TF	Human cell lines	TF	Mouse cell lines
ATF2	GM12878	**BHLHE40**	CH12
CCNT2	K562	**CHD2**	CH12
CDP	GM12878	**COREST**	CH12
CEBPB	H1-hESC	**ETS1**	CH12
E2F1	Hela-S3	**HCFC1**	MEL
E2F4	K562	**IRF4**	NFS-201
E2F6	H1-hESC,Hela-S3,K562	**JunD**	CH12
ELF1	K562,MCF-7,HeG2	**Mix1**	MEL
ELK1	K562	**MyoD**	C2C12
ELK4	Hela-S3	**Myogenin**	C2C12
ETS1	K562	**TAL1**	Megakaryo
ELK4	Hela-S3	**UBF**	CH12
Egr-1	K562,GM12878	**USF2**	CH12
FOXM1	GM12878	**ZNF-MIZD-CP1**	CH12
GABP	A549,GM12878,Hela-S3,HepG2, K562,MCF-7, SK-N-SH	**c-Jun**	CH12
HA-E2F1	Hela-S3,MCF-7	**c-Myc**	CH12
HDAC2	MCF-7	**p300**	CH12
HMGN3	k562	**Med1**	MEF
IRF4	GM12878		
MTA3	GM12878		
MAX	HCT116, NB4		
Mxi1	Hela-S3		
NFIC	HepG2		
NFKB	GM12892, GM15510, GM18951,GM19099,GM19193		
Nrf1	GM12878,H1-hESC,Hela-S3, HepG2, K562, SK-N-SH		
PAX5-C20	GM12878		
PML	GM12878		
PU.1	GM12878,GM12891,HL-60, K562		
Pbx3	GM12878		
Rad21	IMP90		
STAT5A	GM12878		
Sin3Ak-20	A549, GM12878, H1-hESC, HepG2, K562		
TAF1	GM12891,GM12892,K562, SK-N-SH		
TBP	K562		
TCF7L2	GM12878,HCT116, PANC-1		
THAP1	K562		
USF-1	ECC-1		
YY1	ECC-1,SK-N-SH		
ZC3H11A	K562		
c-Fos	MCF10A		
c-Myc	MCF-7		

A549: human lung adenocarcinoma epithelial cell line; **C2C12**: myoblast cell line derived from thigh muscle of C3H mice after crush injury; **CH12**: mouse B-cell lymphoma; **ECC1**: endometrium adenocarcinoma; **GM(#):** epstein-Barr Virus transformed B-lymphocyte, lymphoblastoid; **H1-hESC:** human embryonic stem cell; **Hela-S3:** cervical carcinoma; **HepG2:** hepatocellular carcinoma; **HCT116:** colorectal carcinoma; **K562:** myelogenous leukemia; **HL-60**: human promyelocytic leukemia cells; **IMP90**: the human diploid fibroblast strain; **MCF7:** mammary glad, adenocarcinoma; **MCF10A:** mammary gland, non-tumorigenic epithelial, inducible cell line; **MEF:** mouse embryonic fibroblast; **MEL:** mouse erythroleukemia; **NB4**: acute promyelocytic leukemia cell line; **NFS-201**: mouse B cell lymphoma; **Megakaryo:** megakaryocyte (primary cells); **PANC-1**: pancreas epithelioid carcinoma; **SK-N-SH**: neuroblastoma cell line differentiated with retinoid acid.

1.1.5.2 Post-translational regulation

Protein post-translational modifications (PTMs) comprise mainly phosphorylation, acetylation, oxidation, methylation, ubiquitylation and sumoylation[116]. PTMs can be either reversible or irreversible and often serve as on and off switches or modulators of protein activity and targeting. PTMs also regulate the assembly and disassembly of protein–protein and protein–nucleic-acid interactions[117]. Among them, protein phosphorylation that usually occurs on serine, threonine and tyrosine residues is the most common type of PTM[118]. Phosphorylation at different sites on ARE-BPs have been shown to control their subcellular distribution or affect their binding affinity[119–124].

We identified Wig-1 PTMs by immunoprecipitation of Flag-Wig-1 protein in cisplatin treated Saos-2 cells followed by mass spectrometry (MS). A number of PTMs of Wig-1, including phosphorylation, acetylation and methylation were identified. Interestingly, Wig-1 PTM changed upon treatment with cisplatin, suggesting that Wig-1 might have different activities and regulate different targets in non-stressed cells as compared to stressed cells.

1.1.6 Wig-1 and cervical carcinoma

1.1.6.1 Wig-1 alterations in cancer

Human *WIG-1* is localized to chromosome 3q26.32[41], a region that contains several genes with relevance to cancer. Gain of 3q region with a minimal common region at 3q26.1-27 has been reported in lymphoma[125], squamous lung carcinoma[126,127] and other cancer types[128]. Online databases show that *WIG-1* is commonly deregulated, amplified or mutated in cancer. Analysis of the in silico transcriptomics database (IST) and the gene expression Atlas shows that *WIG-1* is amplified in various tumor types including AML, glioma and lung cancer (http://ist.medisapiens.com/, www.ebi.ac.uk/gxa). According to cBioPorta that with exploration and analysis of the Cancer Genomic Atlas (TCGA) data, *WIG-1* is commonly amplified in many tumors, including 54 % of lung squamous cell carcinomas, 28% of ovarian cancer, 24% of breast cancer, 21% head& neck cancer, 18% of Esophagus cancer and 18% cervical carcinomas (www.cbioportal.org).

1.1.6.2 Cervical carcinoma

Cervical carcinoma arises from the uterine cervix and ranks as the third most common type of cances in women worldwide (following breast and colorectal cancer)[129]. National estimates of 5-year survival of cervical cancer range from less than 50% to more than 70%, according to the global surveillance of cancer survival study[130]. Although the incidence and mortality rates of cervical carcinoma have dropped substantially in developed countries after the high-coverage screening[131–134], this disease remains a serious health threat in places with poor health care such as Eastern Africa, South-Central Asia and Melanesia[135].

Two major types are squamous cell carcinoma (SCC), accounting for around 80% of the cases, and adenocarcinoma (ADCA) that accounts for the remaining 20%[136]. SCC derived from the flat, skin-like epithelial cells that cover the outer surface of the cervix (the ectocervix) while the ADCA is derived from glandular cells on the body of the uterus (the endocervix). Both types of cancer share the same major risk factor, namely infection with high-risk (HR) human papillomavirus (HPV)[137], with HPV 16 and HPV 18 as the two predominant types[135,138].

Most HPV infections are cleared within two years[139], but with persistent infections, a low grade lesion can progress to pre-cancer and further invasive cancer[135]. HPVs affect two major cellular tumor suppressor pathways by encoding two early proteins, E6 and E7. E6 interacts with p53 tumor suppressor and targets it for degradation, disrupting p53-dependent apoptosis and/or senescence pathways[140,141]. E7 binds to and blocks another tumor suppressor, the retinoblastoma protein (pRB), thereby interfering with G1/S transition control in the cell cycle[142,143]. Consequently, HPV infection may lead to malignant transformation. Therefore, the screening with test for HPV DNA is the most effective way to detect early stage infection and to prevent the progression of invasive cervical cancer[144,145].

This is not the end of story for curing cervical cancer patients. HPV-negative cervical carcinoma is associated with different risk factors[146] and has a poor prognosis compared to HPV-positive cervical carcinoma[147–149]. Increased mortality in HPV-negative patients might be due to mutation of important tumor suppressor genes such as p53 and RB, but

studies have shown that there is no clear correlation between p53 mutation and development of HPV-negative cervical carcinoma, indicating that additional mechanisms, as yet unidentified, might be involved[150].

1.1.6.3 Wig-1 expression in cervical carcinoma

Genomic alterations such as gene amplifications are key features of cervical carcinogenesis[151]. Gain of copy number of the long arm of chromosome 3 serves as a biomarker for progression from cancer in situ (CIN) to invasive carcinoma[152]. Genes localized to the 3q26 region, including the phosphoinositide-3-kinase catalytic alpha polypeptide gene (*PIK3CA*)[151] and the telomerase RNA component gene (*TERC*) [153,154]. However, no previous study has been carried out to investigate whether *WIG-1* gene, which is also localized this region, contributes to cervical carcinogenesis. Analysis of TCGA data revealed that *WIG-1* is amplified in 35 cases (18%) of the total 191 cervical squamous cell carcinoma and endocervical adenocarcinoma cases that were studied.

1.1.7 Wig-1 and stem cells

1.1.7.1 The early embryonic development

Mammalian embryogenesis initiates with the fertilization of an egg cell (oocyte) by a sperm cell. Once fertilized, the cell containing two sets of chromosomes (diploid) is called a zygote. The zygote undergoes several divisions to form 2 cells and then 4 cells, followed by 8 cells, 16-32 cells (also called morula because of the mulberry shape), 64 cells and then 128 cells. At the 64 and 128-cell stages, this cluster of cells is also called blastocyst, from a greek word meaning "a sprout". The blastocyst comprises an inner cell mass (ICM) that will grow into an embryo and the outer layer called trophectoderm, which will form the placenta and initiate the implantation in the uterus[155]. Cells derived from the inner cell mass are pluripotent and are called embryonic stem cells[156,157]. They are widely used in scientific research and has also been applied in clinical trial[158].

The formation of the blastocyst before implantation is called the early embryonic development stage. In the mouse, it takes about 3-4 days and in human 5 days (Figure 5). The gene regulation at this early embryonic stage can be separated into two phases: maternal control and zygotic control. Maternal control means that maternal RNAs

existing in the egg are being used to drive early development. These RNAs are degraded gradually during embryogenesis[159]. Zygotic control means that zygotic transcripts are expressed and drive embryonic development from the 2-cell stage in the mouse[160–162] and from the 4 to 8-cell stages of human development[163].

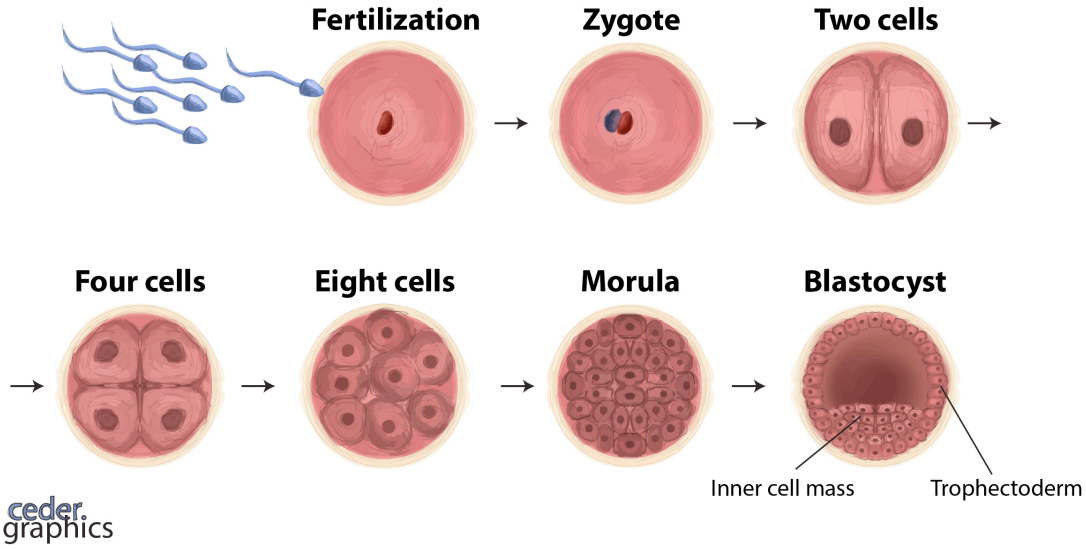


Figure 5. The early embryonic development stages (figure by Sophia Ceder, link: www.ceder.graphics)

1.1.7.2 Wig-1 expression during early embryogenesis

`Wig-1 maternal mRNA expression is already detectable at the mid-2 cell stage after fertilization but drops at the late-2 cell stage. At that time, zygotic transcription kicks in, leading to a rapidly elevated Wig-1 mRNA expression at the 4 cell stage and a peak at 8 cell stage, followed by stable expression levels until the late blastocyst stage[164].

1.1.7.3 Wig-1 expression in stem cells

Wig-1 was found to be upregulated in stem cells including haematopoietic, neuronal and ESCs as compared to their corresponding differentiated cell types[165]. Another study showed increased Wig-1 expression in bone marrow cells lacking the proto-oncogene Bmi1, a polycomb group repressor, which is essential for haematopoietic stem cell (HSCs) self-renewal[166]. An shRNA screen to find novel ESC regulators showed that Wig-1 silencing by shRNA results in reduced hESCs identity[167]. The role for Wig-1 in

ESC maintenance is supported by the observation that Wig-1 mRNA expression levels are gradually decreased as mESCs undergo differentiation[168]. Furthermore, Wig-1 was also shown to be one of the novel RNA-binding proteins in the mESC mRNA interactome[169].

All of there above suggests that Wig-1 may be important for the maintenance of ESCs and embryonic development.

1.1.8 The Myc oncogene

Myc was originally identified as a viral oncogene, v-Myc, in MC29, a virus that induces myelocytomatosis and other neoplastic diseases in chickens[170,171]. c-Myc, the cellular homolog of v-Myc and the founding member of the Myc family, was characterized[172] and found to be frequently rearranged and overexpressed in Burkitt lymphoma and murine plasmacytoma[8,9]. The other two family members, *MYCN*[173,174] and *MYCL*[175], were later identified as amplified oncogenes in neuroblastoma and lung cancer.

Indeed, about 50% of human cancers show gene amplification and/or overexpression of *MYC*/Myc[176] (www.cbioportal.org). c-Myc/N-Myc/L-Myc have distinct expression patterns in adult tissues[177]. Overexpression of c-Myc is common in both blood-borne and solid tumors. N-Myc is most often overexpressed in cancers of neural origin, such as glioma and neuroblastoma[178], while *MYCL* gene amplification is frequently observed in small cell lung cancer[175].

1.1.8.1 Role of Myc as transcription factor

The Myc family proteins are basic-helix-loop-helix leucine-zipper (bHLH-LZ) transcriptional factors in which the bHLH functions as DNA-binding domain while the LZ forms a heterodimers with another bHLH-LZ protein named Max[179]. In most cases, Myc-Max heterodimers bind directly to the E-box sequence (CACGTG) of the target genes. Myc-Max regulates a wide range of genes, participating in genomic stability, cell cycle progression, apoptosis, differentiation and metabolism (reviewed in[180–182]). In addition, Myc can also indirectly regulate mRNAs stability via ARE-BPs. Myc represses

transcription of TTP, one of the ARE-BPs that participate in the degradation of ARE-containing mRNAs[183].

1.1.8.2 Myc role in stem cell and embryonic development

All three Myc family members are abundantly expressed throughout embryonic development[184], and targeted deletion of c-Myc or N-Myc in mice causes embryonic lethality [185–191] while silencing of L-Myc leads to no detectable phenotype[192]. c-Myc knockout mice die before E10.5 with hematopoietic and vascular defects[189]. The dose of N-Myc is important for the severity of the phenotype of N-Myc knock out embryos (reviewed in [193]). N-Myc null mutations result in embryonic lethality at E11.5, with defects in heart, liver, stomach, lung, kidney, and nervous systems development[186,188,191]. On the other hand, N-Myc hypomorphic mutants and a compound heterozygous genotype which still show approximately 15% expression levels of N-Myc protein, result in a significantly longer survival time of the embryos and more-restricted effects on organs such as lung and heart[187,190,194] compared to N-Myc null knockouts.

The observation that c-Myc or N-Myc deficient mouse embryos survive until midgestation (E9-E11) can be explained by the fact Myc gene family expression patterns become most divergent at that time. Indeed, knockout of their common obligate partner Max is embryonic lethal at an earlier embryonic stage E6.5[195]. Additionally, it has been shown that N-Myc can rescue the essential role of c-Myc in mouse embryonic development, cellular survival and differentiation [196].

c-Myc is also crucial for maintenance of embryonic stem cell (ESC) pluripotency. It sustains stem cells self renewal by blocking differentiation independent of LIF expression[197]. Later on, c-Myc was identified as one of "magic four" genes for reprogramming fibroblasts into induced pluripotent stem cells (iPS) [198–201].

Double knockout of N-Myc and c-Myc in ESC causes growth inhibition due to cell cycle arrest and an increase in apoptosis as well as differentiation into ectoderm, mesoderm, and endoderm derivatives. Chimeric embryos injected with double knock out mESCs mostly failed to develop and or in same rare cases when they did form, they had very severe defects[202].

Taking all this together, both c-Myc and N-Myc are key regulators of embryogenesis and variations in Myc expression levels have important consequences. Myc also plays crucial roles in the maintenance of pluripotency and self-renewal capacity of stem cells.

1.1.8.3 Regulation of Myc by Wig-1

Both N-Myc and c-Myc can be regulated by ARE-BPs[203,204] or co-regulated by ARE-BPs and microRNAs[205]. HuR, a well studied ARE-BP, reduces c-Myc expression by recruiting miRNA let-7-associated RISC to the 3'UTR of c-Myc mRNA[205]. Two other ARE-BPs: AUF1 and TIAR affect c-Myc mRNA translation through an ARE and this regulation is a dynamic process dependent on the ratio of AUF1 and TIAR bound to c-Myc mRNA[206].

In conclusion, it is perhaps not surprising that N-Myc is also regulated by Wig-1 since we had identified Wig-1 as an ARE-BP. Wig-1 stabilizes N-Myc mRNA via binding to its proximal ARE in the 3'UTR, thereby promoting N-Myc-driven tumor growth. Silencing of Wig-1 leads to differentiation of neuroblastoma cells carrying amplified N-Myc[42].

1.2 AIMS OF THIS THESIS

The overall aims were to identify Wig-1 mRNA targets and investigate the biological implications of the regulation of selected targets. We also wished to evaluate Wig-1 protein expression in tumors and its correlation to patient survival, and determine the role of Wig-1 in mouse development.

Paper I: To study the effects of Wig-1 knockdown on global gene expression and cell survival. To explore the mechanisms of Wig-1-mediated regulation of the pro-apoptotic FAS at the mRNA level.

Paper II: To investigate Wig-1 protein expression in cervical carcinoma and study a possible association between Wig-1 expression and patient survival.

Paper III: To understand the role of Wig-1 in mouse development and why complete lack of Wig-1 causes embryonic lethality.

Paper IV: To characterize the mRNA-binding properties of Wig-1 and identify RNA secondary structures involved in Wig-1 mRNA binding on a transcriptome-wide scale.

1.3 RESULTS AND DISCUSSION

Paper I

Wig-1 regulates cell cycle arrest and cell death through the p53 targets FAS and 14-3-3σ

Previously, Wig-1 has been shown to bind to and regulate p53[109] , N-Myc[42] and p21 mRNAs[207]. In this study, our goal was to find out other novel Wig-1 target mRNAs.

We first applied microarray analysis to identify Wig-1 target mRNAs by knocking down Wig-1 in HCT116 colon cancer cells. Wedentified 2447 transcripts with >4-fold changes of expression levels between Wig-1 and control siRNA-treated cells. Using the PANTHER software, we analyzed significantly deregulated pathways after Wig-1 knockdown with Alzheimer's and Huntington's diseases, the p53 pathway, the FAS signaling pathway and apoptosis among the top ranked pathways.

Eight targets (*FAS*, *WNT1*, *FZD8*, *AKT3*, *APP*, *14-3-3σ*, *CDC42*, *PPP2CB*) were selected for validation based on the extent of expression changes after Wig-1 knockdown (KD). Changes in protein levels for *FAS*, *WNT1*, *AKT3*, *APP*, *14-3-3σ*, and *PPP2CB* were consistent with the microarray data.

We moved on to study how Wig-1 regulates the targets FAS and 14-3-3*σ* since: 1) both of them are p53 targets and 2) the p53 pathway is one of the most affected pathways after Wig-1 KD. We found that Wig-1 binds to and destabilizes FAS mRNA through its ARE in the 3'UTR. 14-3-3*σ*, which does not have any AREs is regulated by Wig-1 positively at the level of transcription. We also found that this regulation is p53- independent since Wig-1 regulates FAS and 14-3-3*σ* in both p53 wild type and p53 null HCT116 cells.

As FAS is a proapoptotic factor and 14-3-3*σ* is involved in cell cycle arrest, we then examined if Wig-1 plays a role in controlling the outcomes of cellular stress. We observed that Wig-1 KD leads to increased cell death and reduced cell cycle arrest upon cellular stress (cisplatin and gamma radiation). In addition to cancer cells, we also

examined Wig-1 knockdown in primary human fibroblasts (HDFs) with or without gamma irradiation. Our data demonstrate that Wig-1 promotes cell cycle arrest and therefore leads to long-term cell survival upon gamma radiation.

How does Wig-1 regulate *FAS* mRNA stability? Is the ARE-mediated mRNA decay pathway involved? Indeed, we found that Wig-1 colocalizes and interacts with CNOT6, a component of CCR4–NOT, suggesting that Wig-1 might be the connecting factor between FAS mRNA and the deadenylase machinery. Furthermore, immunofluorescence and RNA-FISH revealed that Wig-1, CNOT6 and FAS mRNA all localize in stress granules after arsenite stress.

To sum up, our results suggest that, in response to cellular stress, Wig-1 acts as a survival factor promoting cell cycle arrest rather than cell death through the regulation of FAS and 14-3-3α mRNA. We also suggest a mechanism through which Wig-1 can destabilize its target mRNAs acting as a bridge between the deadenylase complex and its mRNA targets in stress granules (Figure 6). This prosurvival function of Wig-1 is also supported by paper II and paper III.

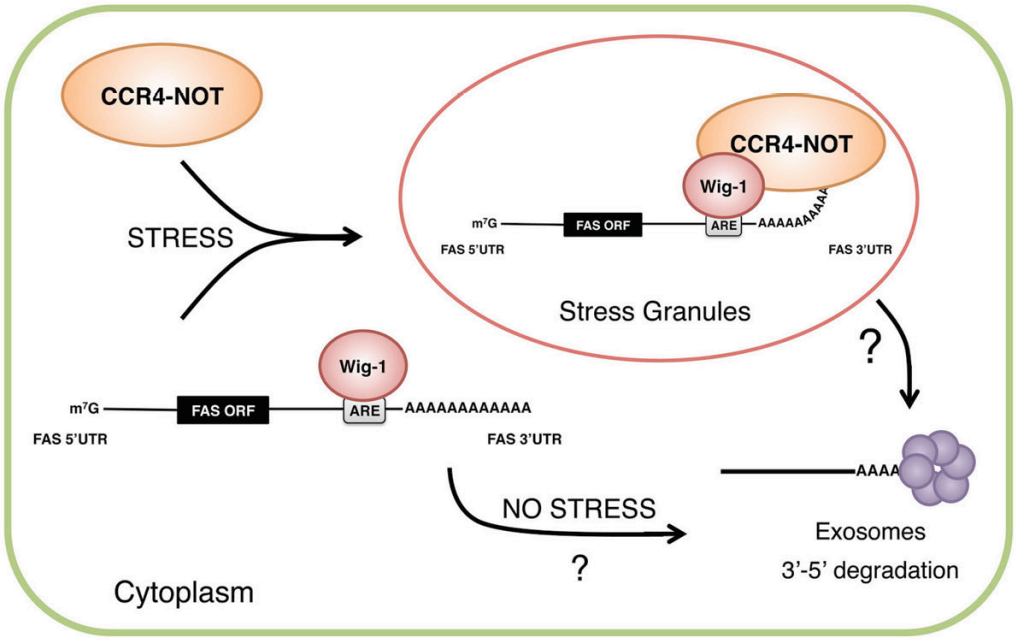


Figure 6: The proposed regulatory model of FAS by Wig-1. (Bersani et al.,Oncogene, 2014)

Paper II

Expression of the p53 target Wig-1 is associated with HPV status and patient survival in cervical carcinoma

Paper I revealed that the p53 target Wig-1 has a prosurvival function. As discussed in the introduction, the *WIG-1* gene is localized to chromosome 3q26, a region that is amplified in many cancer types. Additionally, as revealed by the cancer genomic data, the *WIG-1* gene is amplified in many tumors, including half of lung squamous cell carcinomas, and around 20% of ovarian cancer, breast cancer and cervical carcinomas (www.cbioportal.org). These results promoted us to study the clinical relevance of Wig-1. Therefore, we examined Wig-1 expression in patient samples for the first time and explored a possible prognostic value of Wig-1.

We initially examined structural and copy number alterations of the *WIG-1* locus in eight cervical carcinoma cell lines (Ca Ski, C-4I, C-33A, SiHa, SW756, MS751, ME-180, and HT-3) by spectral karyotype and comparative genomic hybridization, respectively. We found amplifications in Ca Ski, ME-180 and SiHa cells that involved 3q23–26, 3q27-ter and 3q23–24, respectively. We then studied possible alterations of *WIG-1* in these cells by Southern blotting analysis. This showed modest gains of *WIG-1* in MS751 and ME-180 cells, but not in the other cells. We concluded that 3q amplifications occur in cervical cancer cells, but the amplifications are not driven by Wig-1. Additionally, Northern blotting and qRT-PCR demonstrated that Wig-1 mRNA levels were higher in the HPV-negative cervical cancer cell lines than in the HPV-positive lines. However, we found no association between Wig-1 protein expression and HPV infection.

We then assessed Wig-1 expression by immunohistochemistry (IHC) in a series of 38 cervical tumor samples comprising both adenocarcinomas and squamous carcinomas in collaboration with Prof. Sonia Andersson at the Dept. of Women's and Children's Health, Astrid Lindgren Hospital. We observed higher nuclear Wig-1 expression levels in HPV-negative cases compared to HPV positive cases ($p=0.002$), suggesting that elevated Wig-1 expression might contribute to cervical carcinogenesis in the absence of HPV infection. This is consistent with the finding that HPV-negative cervical cancer cell lines have higher Wig-1 mRNA expression levels compared to the HPV-negative lines.

We also noted that Wg-1 expression levels are significantly higher in adenocarcinomas as compared to squamous cell carcinomas ($p<0.0001$). Most remarkably, we observed several distinct Wig-1 staining patterns in cervical tumors and found that patients with moderate nuclear Wig-1 staining and positive cytoplasmic Wig-1 staining in their tumors had longer survival than patients with strong nuclear and negative cytoplasmic staining ($p=0.042$) (Figure 7).

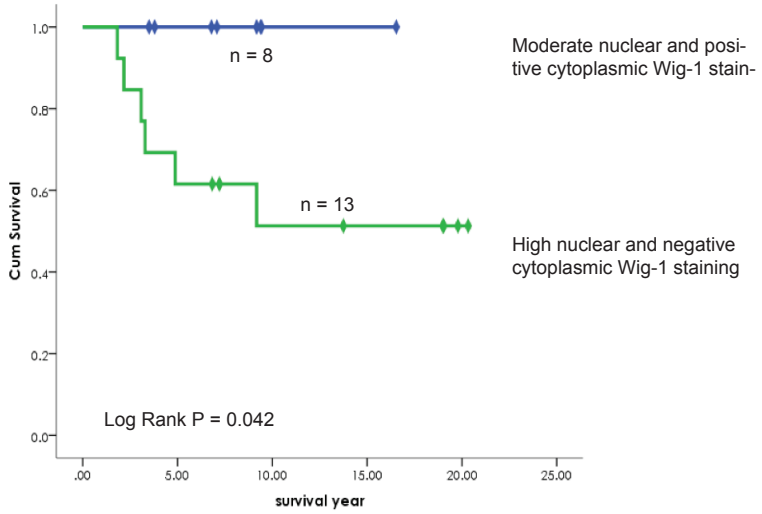


Figure 7: A. Kaplan-Meier survival curve showing that patients with moderate nuclear and positive cytoplasmic Wig-1 staining have better survival than patients with strong nuclear and negative cytoplasmic staining ($p < 0.05$ is considerate to be significant). (Xu et al., PLoS ONE, 2014)

What is the biological significance of this association? Our previous findings indicate that Wig-1 exerts a growth-promoting and/or anti-cell death function by upregulating putative targets such as N-Myc and downregulating the pro-apoptotic FAS[26,74] (Paper I). Therefore, it is conceivable that high nuclear Wig-1 expression in cervical cancer cells drives cell proliferation through stabilization of pro-growth mRNA targets while destabilizing of the pro-apoptotic mRNA targets. However, the exact roles of nuclear and cytoplasmic Wig-1 need to be studied further.

To conclude, our finding that Wig-1 expression elevated in HPV-negative cervical carcinoma compared to HPV-positive samples suggests a possible role of Wig-1 in HPV-negative cervical carcinogenesis. Moreover, our data demonstrate that moderate nuclear Wig-1 expression levels and positive cytoplasmic Wig-1 staining are associated with better prognosis, suggesting that Wig-1 protein expression levels assessed with IHC could serve as a novel molecular marker or a molecular marker combined with other traditional clinical markers for prognosis of cervical cancers. Our results may contribute to a better understanding of the molecular basis of carcinogenesis.

Paper III

Complete lack of Wig-1 leads to embryonic lethality before the blastocyst stage

The involvement of Wig-1 in proliferation, senescence and differentiation has naturally raised the question whether Wig-1 is important for embryonic development. To address this, we set out to investigate the role of Wig-1 during mouse embryogenesis.

First, we studied Wig-1 expression in mouse embryos. We found that Wig-1 mRNA is expressed at high levels at E5.5 as shown by Northern blotting. Levels then drop somewhat to peak again at E10.5-13.5. The expression then decreases markedly. We also noticed that Wig-1 protein expression levels are higher in mouse embryonic stem cells (mESC) compared to differentiated ESCs. Furthermore, Wig-1 expression is independent of p53, at least at E10.5 and E13.5, as indicated from IHC of Wig-1 in p53 knockout embryos.

We then generated mice carrying inactivated Wig-1 alleles by targeted deletion of parts of exon 2 to exon 4. Intercrossing of heterozygous Wig-1 mice with wild type mice, revealed a reduced frequency of heterozygous offspring (12% instead of the expected 50%) but no other obvious phenotypic abnormalities. Furthermore, we failed to detect any Wig-1 null offspring after intercrossing heterozygous mice with each other, indicating that complete lack of Wig-1 is associated with embryonic lethality. To find out if the Wig-1 null embryos were present at blastocyst stage, we performed *in vitro* fertilization (IVF) and cultured zygotes until the blastocyst stage. Still, we were not able

to detect any Wig-1 null blastocysts, suggesting that Wig-1 null embryos die before the blastocyst stage.

Next, we asked why complete lack of Wig-1 leads to embryonic lethality. We examined cellular processes that might be affected by Wig-1 KD in mESCs. We found that Wig-1 KD causes a significant reduction in cell proliferation as demonstrated by cell count and WST-1 assays, a colorimetric assay for quantification of cellular proliferation and viability. Reduced proliferation in the absence of Wig-1 could lead to impaired embryonic development, especially in the early pre-implantation embryo.

Since we have shown that Wig-1 is an ARE-BP, we speculated that the early lethality of Wig-1 null embryos could be due to deregulation of Wig-1 targeted mRNAs. We have already found that Wig-1 regulates N-Myc mRNA via AREs and that Wig-1 KD leads to decreased N-Myc mRNA and N-Myc protein levels[42]. Both c-Myc and N-Myc seem to be required for early embryonic development[209,210] and maintenance of stem cell survival and self-renewal[202]. Therefore, we hypothesized that the lethality of Wig-1 embryos could be due to altered expression of c-Myc and N-Myc.

To test this hypothesis, we examined Wig-1 in mESCs. Wig-1 siRNA KD caused attenuated c-Myc and N-Myc protein expression did not affect mRNA levels, suggesting that Wig-1 regulates Myc at the translational level. Moreover, we found that Wig-1 binds both c-Myc and N-Myc mRNA. These results indicate that the embryonic lethality in the absence of Wig-1 is at least in part due to insufficient Myc expression prior to the blastocyst stage.

Given that Wig-1 binds to as many as 286 mRNAs (paper IV) and regulates 2447 targets either directly or indirectly (paper I), it is likely that Wig-1 knockout affects a number of targets that contribute to the embryonic lethality. However, the combined effect of reduced expression of c-Myc and N-Myc – two key regulators of cell survival and proliferation – by Wig-1 may contribute in a significant way to the phenotype observed in Wig-1 knockout mice independently of effects on other targets. Nonetheless, it is likely that other Wig-1 targets are also important for the early embryonic lethality.

In summary, Wig-1 is expressed in the brain, spinal cord, and fetal liver in C57Bl/6 embryos in a p53-independent manner. Loss of Wig-1 leads to embryonic lethal, most probably due to dysregulation of the Wig-1 targets N-Myc and c-Myc during early embryonic development. A model for Wig-1 regulation of Myc during early embryonic development is shown in Figure 8.

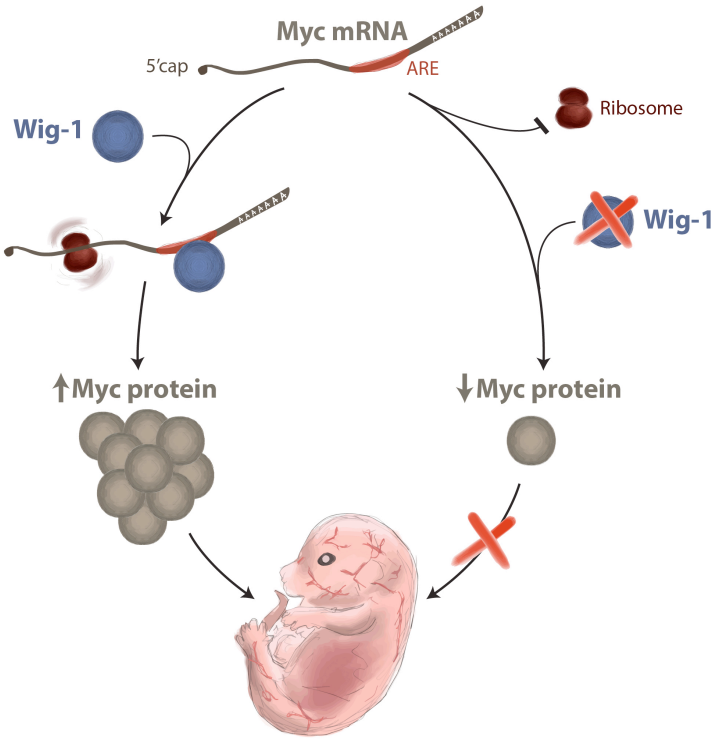


Figure 8. Model for Wig-1 regulation of Myc during embryonic development. AREs in the 3'UTR of Myc mRNA regulate its translation. Wig-1 binds the Myc AREs and recruits ribosomes to initiate translation of Myc mRNA. Myc protein can then transactive its target genes and promote cell growth that required for normal embryonic development. However, if Wig-1 expression is shut down, Myc mRNA translation will be abolished or attenuated, resulting in impaired Myc expression and embryonic lethality.

Paper IV

Genome-wide identification of Wig-1 mRNA targets by RIP-Seq analysis

The aim of this study was to identify novel Wig-1-associated mRNAs. We performed RNA-immunoprecipitation followed by high-throughput sequencing (RIP-Seq) in both HCT116 and Saos-2 cells, which resulted in a list of 286 Wig-1-bound mRNAs common between both cell lines. By putting all the Wig-1 bound mRNAs into network enrichment analysis (NEA), we showed that Wig-1 targets are highly connected with the Cell Cycle pathways, in accordance with paper I.

For validation, we selected nine Wig-1-associated mRNAs out of the 286 mRNA that are enriched in both HCT116 and Saos2 cells: *MAD2L1*, *MTHFD2*, *CCNG1*, *EIF4E*, *CHEK1*, *RMI1*, *HIF1A*, *AMD1* and *CAV1*. By RNA-IP followed with qRT-PCR, we validated all nine targets in HCT116 and 6 of the targets in Saos2 cells. Additionally, we found that Wig-1 knockdown in HCT116 cells decreased the expression levels of *MTHFD2*, *EIF4E*, *RMI1* and *CAV1* mRNA but led to an increased level of *HIF1A* mRNA. These findings demonstrate that Wig-1 can both stabilize and destabilize its RNA targets, including pro-and anti-proliferation factors.

We further examined the features of Wig-1-bound mRNAs. Sequence analysis revealed that AREs and/or generally AU-rich motifs are highly enriched in the 3'UTR of these mRNAs. Secondary structure analysis identified a shared consensus 2D motif among the nine validated targets.

To summarize, our findings in this study are consistent with our previous data on Wig-1 as an ARE-BP that regulates cell cycle and cell death-related processes. In addition, the present study provides a more comprehensive picture of preferred Wig-1 binding motifs and significantly extends the repertoire of Wig-1 target mRNAs.

1.4 CONCLUSION AND FINAL DIRECTIONS

Coming back to our first question in the Introduction: What is Wig-1? What is the cellular function(s) of Wig-1? I hope I have managed to tell you a good story of Wig-1 and inspired you to develop some interest in Wig-1 now. Compared to p53 which has more than 77, 000 publications indexed to PubMed from 1979, Wig-1 has so far only around 50 publications in total since its discovery in 1997, leaving us much to do in the field of research on Wig-1.

Wig-1 is one of the p53 targets, whose RNA and protein expression levels increase after p53 activation. Both human and mouse Wig-1 have p53 response elements.

Wig-1 is an AU-rich element-binding protein (ARE-BP) that regulates its target mRNAs at the post-transcriptional level via direct binding to AREs. Through paper I and paper IV in this thesis, we have extended the list of published Wig-1 targets from p53, N-Myc and p21 to FAS and 14-3-3σ, both downstream targets of p53, and possibly other genes with relevance for cancer, like MTHFD2, RMI1, CAV1 and EIF4E. Interestingly, EIF4E, the eukaryotic translation initiation factor 4E, is a key player in translational control[211,212] by recognizing 5'cap of mRNAs. EIF4E has been shown to be bound and stabilized by HuR via an ARE in the 3'UTR[213]. In paper IV, we showed Wig-1 binds to and stabilizes EIF4E mRNA, which may contribute to EIF4E mediated translation. If further study can show that Wig-1 knockdown affect N-Myc translation via downregulating EIF4E, this will support our proposed model for Wig-1 regulation of Myc at the translational level during embryogenesis, as shown in paper III (Figure 8).

Although we have shown that Wig-1 can regulate mRNAs by affecting their stability[42,109] (paper I) or possibly translation (paper III), we are still open to other possible functions of Wig-1 at various levels of gene regulation, such as alternative splicing and mRNA export from nucleus to cytoplasm, as for other ARE-BPs. We also believe that Wig-1 affects cell growth more than by just preventing apoptosis or senescence[110]. Indeed, data from our lab suggest that Wig-1 can affect the cellular redox system (S. Eriksson, unpublished results).

Moreover, we have also noted that Wig-1 binds and destabilizes HIF1A mRNA (paper IV). Previous studies have shown that p53 negatively regulates both HIF1A transcription and protein levels[214]. Our data suggest that p53 regulates HIF1A through Wig-1. As discussed in the Introduction, p53-mediated cell cycle arrest, apoptosis and senescence do not appear to be crucial for p53-mediated tumor suppression[36,37]. Li et al., have shown that p53 inhibits cysteine uptake and sensitizes cells to ROS-induced cell death [215]. Therefore, It would be interesting to examine if Wig-1 pays also a role in the regulation of hypoxia-induced p53-dependent apoptosis or metabolic regulation.

Paper II concludes that Wig-1 expression patterns in tumor samples are associated with prognosis. Patients with moderate nuclear Wig-1 expression and positive cytoplasmic Wig-1 expression in their tumors have better survival compared to patients with high nuclear Wig-1 expression and negative cytoplasmic Wig-1 expression. This finding implies that the Wig-1 protein might serve as a molecular marker together with other conventional clinical markers for prognosis of cervical cancer. Indeed, Wig-1 expression is associated with chemosensitivity, overall survival and clinical stage in small cell lung cancer (SCLC). Wig-1 silencing reduced SCLC resistance to several drugs including cisplatin, doxorubicin and etoposide by increasing apoptosis[216]. Moreover, Wig-1 knockdown was shown to sensitize human lymphoblastoid cells (LCLs) and non-small cell lung cancer cells (A549) to pemetrexed treatment[217]. From a therapeutic point of view, we believe that further studies need to be carried out to investigate the clinical significance of Wig-1 expression. We also believe that full understanding of how Wig-1 regulates its target mRNAs will serve to develop potential diagnostic and therapeutic strategies against cancer.

Paper III shows that Wig-1 null embryos die before the blastocyst stage. Intercrossing of Wig-1 heterozygous (wig-1+/-) mice with Wig-1 wt (Wig-1+/+) mice revealed a skewed ratio between Wig-1 +/+ and Wig-1 +/- offsprings (88%:12%), which is significantly different from the expected Mendelian ratio (50%:50%). No other obvious phenotypic abnormalities are evident. These findings indicate that Wig-1 is essential for early embryonic development. Since no Wig-1 null mice are born, studies of the effect of complete lack of Wig-1 in adult tissues must be carried out using conditional knock out

systems. We are currently in the process of generating such mice. They can be used to investigate tumor formation in tissues lacking Wig-1 (e.g. lung or cervix) and hopefully provide information about the exact roles of Wig-1 in tumor development. We should also achieve a better understanding of the physiological functions of Wig-1 in specific tissues.

Altogether, we believe that Wig-1 is a pro-survival factor and a critical regulator of embryonic development. In addition, Wig-1 may be of great significance in cancer development and therapy resistance.

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3 REFERENCES

1 American Cancer Society. Global Cancer Facts & Figures 3rd Edition. *Am Cancer Soc* 2015; : 1–64.

2 Hanahan D, Weinberg R a, Francisco S. The Hallmarks of Cancer Review University of California at San Francisco. *Cell* 2000; **100**: 57–70.

3 Hanahan D, Weinberg R a. Hallmarks of cancer: The next generation. *Cell* 2011; **144**: 646–674.

4 Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz L a, Kinzler KW. Cancer Genome Lanscapes. *Science (80-)* 2013; **339**: 1546–1558.

5 Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C *et al.* Mutational landscape and significance across 12 major cancer types. *Nature* 2013; **502**: 333–9.

6 Cancer T, Atlas G. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330–7.

7 Northcott PA, Shih DJH, Peacock J, Garzia L, Sorana Morrissy A, Zichner T *et al.* Subgroup-specific structural variation across 1,000 medulloblastoma genomes. Nature. 2012; **488**: 49–56.

8 Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, Croce CM. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc Natl Acad Sci U S A* 1982; **79**: 7824–7827.

9 Taub R, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S *et al.* Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc Natl Acad Sci U S A* 1982; **79**: 7837–7841.

10 Escot C, Theillet C, Lidereau R, Spyratos F, Champeme MH, Gest J *et al.* Genetic alteration of the c-myc protooncogene (MYC) in human primary breast carcinomas. *Proc Natl Acad Sci U S A* 1986; **83**: 4834–4838.

11 Cher ML, Bova GS, Moore DH, Small EJ, Carroll PR, Pin SS *et al.* Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping. *Cancer Res* 1996; **56**: 3091–3102.

12 Lane DP. Cancer. p53, guardian of the genome. Nature. 1992; **358**: 15–16.

13 Linzer DIH, Levine a. J. Characterization Tumor Antigen and Uninfected of a 54K Dalton Cellular SV40 Present in SV40-Transformed Cells. *Cell* 1979; **17**: 43–52.

14 Lane DP, Crawford L V. T antigen is bound to a host protein in SV40-transformed cells. *Nature* 1979; **278**: 261–263.

15 Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH *et al.* Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990; **250**: 1233–1238.

16 Srivastava S, Zou ZQ, Pirollo K, Blattner W, Chang EH. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature. 1990; **348**: 747–749.

17 Oren M, Rotter V. Mutant p53 gain-of-function in cancer. Cold Spring Harb. Perspect. Biol. 2010; **2**. doi:10.1101/cshperspect.a001107.

18 Zhu J, Sammons M a., Donahue G, Dou Z, Vedadi M, Getlik M *et al.* Gain-of-function p53 mutants co-opt chromatin pathways to drive cancer growth. *Nature* 2015. doi:10.1038/nature15251.

19 Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Butel JS *et al.* Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 1992; **356**: 215–221.

20 Donehower LA, Harvey M, Vogel H, McArthur MJ, Montgomery CA, Park SH *et al.* Effects of genetic background on tumorigenesis in p53-deficient mice. In: *Molecular Carcinogenesis*. 1995, pp 16–22.

21 Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000; **408**: 307–310.

22 Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. *Nat Rev Cancer* 2009; **9**: 749–758.

23 Hainaut P, Wiman KG. 30 years and a long way into p53 research. Lancet Oncol. 2009; **10**: 913–919.

24 Liu Y, Elf SE, Miyata Y, Sashida G, Liu Y, Huang G *et al.* p53 Regulates Hematopoietic Stem Cell Quiescence. *Cell Stem Cell* 2009; **4**: 37–48.

25 Vousden KH, Prives C. Blinded by the Light: The Growing Complexity of p53. *Cell* 2009; **137**: 413–31.

26 Vousden KH, Ryan KM. p53 and metabolism. *Nat Rev Cancer* 2009; **9**: 691–700.

27 Junttila MR, Evan GI. p53--a Jack of all trades but master of none. *Nat Rev Cancer* 2009; **9**: 821–829.

28 Chatoo W, Abdouh M, Bernier G. P53 Pro-Oxidant Activity in the Central Nervous System: Implication in Aging and Neurodegenerative Diseases. *Antioxid Redox Signal* 2011; **15**: 1729–37.

29 El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM *et al.* WAF1, a potential mediator of p53 tumor suppression. *Cell* 1993; **75**: 817–825.

30 Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, Thiagalingam S *et al.* 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. *Mol Cell* 1997; **1**: 3–11.

31 Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell* 2001; **7**: 683–694.

32 Villunger A, Michalak EM, Coultas L, Müllauer F, Böck G, Ausserlechner MJ *et al.* p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science* 2003; **302**: 1036–1038.

33 Müller M, Wilder S, Bannasch D, Israeli D, Lehlbach K, Li-Weber M *et al.* p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. *J Exp Med* 1998; **188**: 2033–2045.

34 Burkhart BA, Alcorta DA, Chiao C, Isaacs JS, Barrett JC. Two posttranscriptional pathways that regulate p21(Cip1/Waf1/Sdi1) are identified by HPV16-E6 interaction and correlate with life span and cellular senescence. *Exp Cell Res* 1999; **247**: 168–175.

35 Adnane J, Jackson RJ, Nicosia S V, Cantor AB, Pledger WJ, Sebti SM. Loss of p21WAF1/CIP1 accelerates Ras oncogenesis in a transgenic/knockout mammary cancer model. *Oncogene* 2000; **19**: 5338–5347.

36 Li T, Kon N, Jiang L, Tan M, Ludwig T, Zhao Y *et al.* Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. *Cell* 2012; **149**: 1269–1283.

37 Valente LJ, Gray DHD, Michalak EM, Pinon-Hofbauer J, Egle A, Scott CL *et al.* p53 efficiently suppresses tumor development in the complete absence of its cell-cycle inhibitory and proapoptotic effectors p21, Puma, and Noxa. *Cell Rep* 2013; **3**: 1339–45.

38 Jiang L, Kon N, Li T, Su T, Hibshoosh H, Baer R *et al.* HHS Public Access. 2015; **520**: 57–62.

39 Varmeh-Ziaie S, Okan I, Wang Y, Magnusson KP, Warthoe P, Strauss M *et al.* Wig-1, a new p53-induced gene encoding a zinc finger protein. *Oncogene* 1997; **15**: 2699–2704.

40 Israeli D, Tessler E, Haupt Y, Elkeles A, Wilder S, Amson R *et al.* A novel p53-inducible gene, PAG608, encodes a nuclear zinc finger protein whose overexpression promotes apoptosis. *EMBO J* 1997; **16**: 4384–4392.

41 Hellborg F, Qian W, Mendez-Vidal C, Asker C, Kost-Alimova M, Wilhelm M *et al.* Human wig-1, a p53 target gene that encodes a growth inhibitory zinc finger protein. *Oncogene* 2001; **20**: 5466–74.

42 Vilborg A, Bersani C, Wickström M, Segerström L, Kogner P, Wiman KG. Wig-1, a novel regulator of N-Myc mRNA and N-Myc-driven tumor growth. *Cell Death Dis* 2012; **3**: e298.

43 Méndez Vidal C, Prahl M, Wiman KG. The p53-induced Wig-1 protein binds double-stranded RNAs with structural characteristics of siRNAs and miRNAs. *FEBS Lett* 2006; **580**: 4401–8.

44 Iuchi S. Cellular and Molecular Life Sciences Three classes of C 2 H 2 zinc finger proteins. *CellMolLife Sci* 2001; **58**: 625–635.

45 Yang M, Stratford May W, Ito T. JAZ requires the double-stranded RNA-binding zinc finger motifs for nuclear localization. *J Biol Chem* 1999; **274**: 27399–27406.

46 Méndez-Vidal C, Wilhelm MT, Hellborg F, Qian W, Wiman KG. The p53-induced mouse zinc finger protein wig-1 binds double-stranded RNA with high affinity. *Nucleic Acids Res* 2002; **30**: 1991–6.

47 Pieper U, Webb BM, Barkan DT, Schneidman-Duhovny D, Schlessinger A, Braberg H *et al.* ModBase, a database of annotated comparative protein structure models, and associated resources. *Nucleic Acids Res* 2011; **39**: D465–D474.

48 Chen CY, Shyu a B. AU-rich elements: characterization and importance in mRNA degradation. *Trends Biochem Sci* 1995; **20**: 465–470.

49 Wilson GM, Brewer G. The search for trans-acting factors controlling messenger RNA decay. *Prog Nucleic Acid Res Mol Biol* 1999; **62**: 257–291.

50 Conne B, Stutz a, Vassalli JD. The 3' untranslated region of messenger RNA: A molecular 'hotspot' for pathology? *Nat Med* 2000; **6**: 637–41.

51 Wu X, Brewer G. The regulation of mRNA stability in mammalian cells: 2.0. *Gene* 2012; **500**: 10–21.

52 Gruber AR, Fallmann J, Kratochvill F, Kovarik P, Hofacker IL. AREsite: A database for the comprehensive investigation of AU-rich elements. *Nucleic Acids Res* 2011; **39**: 66–69.

53 Miller AD, Curran T, Verma IM. C-Fos Protein can Induce Cellular Transformation: a Novel Mechanism of Activation of a Cellular Oncogene. *Cell* 1984; **36**: 51–60.

54 Brewer G. An A + U-rich element RNA-binding factor regulates c-myc mRNA stability in vitro. *Mol Cell Biol* 1991; **11**: 2460–2466.

55 Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S, Cerami A. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc Natl Acad Sci U S A* 1986; **83**: 1670–4.

56 Nair AP, Hahn S, Banholzer R, Hirsch HH, Moroni C. Cyclosporin A inhibits growth of autocrine tumour cell lines by destabilizing interleukin-3 mRNA. *Nature* 1994; **369**: 239–42.

57 Barreau C, Paillard L, Osborne HB. AU-rich elements and associated factors: are there unifying principles? *Nucleic Acids Res* 2005; **33**: 7138–50.

58 Keene JD, Tenenbaum S a. Eukaryotic mRNPs may represent posttranscriptional operons. *Mol Cell* 2002; **9**: 1161–1167.

59 Kishore S, Luber S, Zavolan M. Deciphering the role of RNA-binding proteins in the post-transcriptional control of gene expression. *Brief Funct Genomics* 2010; **9**: 391–404.

60 Brooks S a., Blackshear PJ. Tristetraprolin (TTP): Interactions with mRNA and proteins, and current thoughts on mechanisms of action. *Biochim Biophys Acta - Gene Regul Mech* 2013; **1829**: 666–679.

61 Sanduja S, Blanco FF, Dixon D a. The roles of TTP and BRF proteins in regulated mRNA decay. *Wiley Interdiscip Rev RNA* 2011; **2**: 42–57.

62 Stoecklin G, Colombi M, Raineri I, Leuenberger S, Mallaun M, Schmidlin M *et al.* Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. *EMBO J* 2002; **21**: 4709–18.

63 Trabucchi M, Briata P, Garcia-Mayoral M, Haase AD, Filipowicz W, Ramos A *et al.* The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. *Nature* 2009; **459**: 1010–1014.

64 Briata P, Chen CY, Ramos A, Gherzi R. Functional and molecular insights into KSRP function in mRNA decay. *Biochim Biophys Acta - Gene Regul Mech* 2013; **1829**: 689–694.

65 Brennan CM, Steitz J a. HuR and mRNA stability. *Cell Mol Life Sci* 2001; **58**: 266–277.

66 Gratacós FM, Brewer G. The role of AUF1 in regulated mRNA decay. *Wiley Interdiscip Rev RNA* 2010; **1**: 457–473.

67 Wilson GM, Lu J, Sutphen K, Suarez Y, Sinha S, Brewer B *et al.* Phosphorylation of p40AUF1 regulates binding to A + U-rich mRNA-destabilizing elements and protein-induced changes in ribonucleoprotein structure. *J Biol Chem* 2003; **278**: 33039–33048.

68 Gherzi R, Lee K-Y, Briata P, Wegmüller D, Moroni C, Karin M *et al.* A KH Domain RNA Binding Protein, KSRP, Promotes ARE-Directed mRNA Turnover by Recruiting the Degradation Machinery. *Mol Cell* 2004; **14**: 571–83.

69 Chen CY, Gherzi R, Ong SE, Chan EL, Raijmakers R, Pruijn GJM *et al.* AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell* 2001; **107**: 451–464.

70 Vasudevan S, Steitz J a. AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. *Cell* 2007; **128**: 1105–18.

71 Glisovic T, Bachorik JL, Yong J, Dreyfuss G. RNA-binding proteins and post-transcriptional gene regulation. *FEBS Lett* 2008; **582**: 1977–1986.

72 Baou M, Norton JD, Murphy JJ, Dc W. AU-rich RNA binding proteins in hematopoiesis and leukemogenesis AU-rich RNA binding proteins in hematopoiesis and leukemogenesis. 2011; **118**: 5732–5740.

73 Audic Y, Hartley RS. Post-transcriptional regulation in cancer. *Biol Cell* 2004; **96**: 479–98.

74 Griseri P, Pagès G. Control of pro-angiogenic cytokine mRNA half-life in cancer: the role of AU-rich elements and associated proteins. *J Interferon Cytokine Res* 2014; **34**: 242–54.

75 Kurosu T, Ohga N, Hida Y, Maishi N, Akiyama K, Kakuguchi W *et al.* HuR keeps an angiogenic switch on by stabilising mRNA of VEGF and COX-2 in tumour endothelium. *Br J Cancer* 2011; **104**: 819–29.

76 Wang J, Wang B, Bi J, Zhang C. Cytoplasmic HuR expression correlates with angiogenesis, lymphangiogenesis, and poor outcome in lung cancer. *Med Oncol* 2011; **28**: 577–585.

77 Wang J, Zhao W, Guo Y, Zhang B, Xie Q, Xiang D *et al.* The expression of rna-binding protein hur in non-small cell lung cancer correlates with vascular endothelial growth factor-C expression and lymph node metastasis. *Oncology* 2009; **76**: 420–429.

78 Wang J, Guo Y, Chu H, Guan Y, Bi J, Wang B. Multiple functions of the RNA-binding protein HuR in cancer progression, treatment responses and prognosis. *Int J Mol Sci* 2013; **14**: 10015–10041.

79 Stumpo DJ, Byrd NA, Phillips RS, Ghosh S, Maronpot RR, Castranio T *et al.* Chorioallantoic fusion defects and embryonic lethality resulting from disruption of Zfp36L1, a gene encoding a CCCH tandem zinc finger protein of the Tristetraprolin family. *Mol Cell Biol* 2004; **24**: 6445–55.

80 Beck AR, Miller IJ, Anderson P, Streuli M. RNA-binding protein TIAR is essential for primordial germ cell development. *Proc Natl Acad Sci U S A* 1998; **95**: 2331–6.

81 Katsanou V, Milatos S, Yiakouvaki A, Sgantzis N, Kotsoni A, Alexiou M *et al.* The RNA-binding protein Elavl1/HuR is essential for placental branching morphogenesis and embryonic development. *Mol Cell Biol* 2009; **29**: 2762–2776.

82 Schoenberg DR, Maquat LE. Regulation of cytoplasmic mRNA decay. *Nat Rev Genet* 2012; **13**: 448–448.

83 Von Roretz C, Gallouzi I-E. Decoding ARE-mediated decay: is microRNA part of the equation? *J Cell Biol* 2008; **181**: 189–194.

84 Chen CY a, Shyu A Bin. Mechanisms of deadenylation-dependent decay. *Wiley Interdiscip Rev RNA* 2011; **2**: 167–183.

85 Collart M a., Panasenko OO. The Ccr4-Not complex. *Gene* 2012; **492**: 42–53.

86 Li Y, Kiledjian M. Regulation of mRNA decapping. *Wiley Interdiscip Rev RNA* 2010; **1**: 253–265.

87 Schoenberg DR. Mechanisms of endonuclease-mediated mRNA decay. *Wiley Interdiscip Rev RNA* 2011; **2**: 582–600.

88 Brümmer A, Hausser J. MicroRNA binding sites in the coding region of mRNAs: extending the repertoire of post-transcriptional gene regulation. *Bioessays* 2014; **36**: 617–26.

89 Vasudevan S, Tong Y, Steitz J a. Switching from repression to activation: microRNAs can up-regulate translation. *Science* 2007; **318**: 1931–1934.

90 Fenger-Grøn M, Fillman C, Norrild B, Lykke-Andersen J. Multiple processing body factors and the ARE binding protein TTP activate mRNA decapping. *Mol Cell* 2005; **20**: 905–915.

91 Fabian MR, Frank F, Rouya C, Siddiqui N, Lai WS, Karetnikov A *et al.* Structural basis for the recruitment of the human CCR4-NOT deadenylase complex by tristetraprolin. *Nat Struct Mol Biol* 2013; **20**: 735–9.

92 Young LE, Moore a. E, Sokol L, Meisner-Kober N, Dixon D a. The mRNA Stability Factor HuR Inhibits MicroRNA-16 Targeting of COX-2. *Mol Cancer Res* 2012; **10**: 167–180.

93 Sharma S, Verma S, Vasudevan M, Samanta S, Thakur JK, Kulshreshtha R. The interplay of HuR and miR-3134 in regulation of AU rich transcriptome. *RNA Biol* 2013; **10**: 1283–90.

94 Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W. Relief of microRNA-Mediated Translational Repression in Human Cells Subjected to Stress. *Cell* 2006; **125**: 1111–1124.

95 Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen J *et al.* Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell* 2005; **120**: 623–34.

96 Piecyk M, Wax S, Beck a R, Kedersha N, Gupta M, Maritim B *et al.* TIA-1 is a translational silencer that selectively regulates the expression of TNF-alpha. *EMBO J* 2000; **19**: 4154–4163.

97 Dixon D a, Balch GC, Kedersha N, Anderson P, Zimmerman G a, Beauchamp RD *et al.* Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. *J Exp Med* 2003; **198**: 475–481.

98 Mazan-Mamczarz K, Galbán S, López de Silanes I, Martindale JL, Atasoy U, Keene JD *et al.* RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. *Proc Natl Acad Sci U S A* 2003; **100**: 8354–9.

99 Galbán S, Martindale JL, Mazan-Mamczarz K, López de Silanes I, Fan J, Wang W *et al.* Influence of the RNA-binding protein HuR in pVHL-regulated p53 expression in renal carcinoma cells. *Mol Cell Biol* 2003; **23**: 7083–7095.

100 Katsanou V, Papadaki O, Milatos S, Blackshear PJ, Anderson P, Kollias G *et al.* HuR as a negative posttranscriptional modulator in inflammation. *Mol Cell* 2005; **19**: 777–789.

101 Buchan JR, Parker R. Eukaryotic stress granules: the ins and outs of translation. *Mol Cell* 2009; **36**: 932–41.

102 Stoecklin G, Stubbs T, Kedersha N, Wax S, Rigby WFC, Blackwell TK *et al.* MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. *EMBO J* 2004; **23**: 1313–24.

103 Anderson P, Kedersha N. Visibly stressed: the role of eIF2, TIA-1, and stress granules in protein translation. *Cell Stress Chaperones* 2002; **7**: 213–221.

104 Von Roretz C, Marco S Di, Mazroui R, Gallouzi I-E. Turnover of AU-rich-containing mRNAs during stress: a matter of survival. *Wiley Interdiscip Rev RNA* 2011; **2**: 336–347.

105 Lian XJ, Gallouzi IE. Oxidative stress increases the number of stress granules in senescent cells and triggers a rapid decrease in p21waf1/cip1 translation. *J Biol Chem* 2009; **284**: 8877–8887.

106 Varmeh-Ziaie S, Ichimura K, Yang F, Rabbits P, Collins VP. Cloning and chromosomal localization of human WIG-1/PAG608 and demonstration of amplification with increased expression in primary squamous cell carcinoma of the lung. *Cancer Lett* 2001; **174**: 179–187.

107 Prahl M, Vilborg A, Palmberg C, Jörnvall H, Asker C, Wiman KG. The p53 target protein Wig-1 binds hnRNP A2/B1 and RNA Helicase A via RNA. *FEBS Lett* 2008; **582**: 2173–7.

108 Sedaghat Y, Mazur C, Sabripour M, Hung G, Monia BP. Genomic analysis of wig-1 pathways. *PLoS ONE* 2012; **7**: e29429.

109 Vilborg A, Glahder J a, Wilhelm MT, Bersani C, Corcoran M, Mahmoudi S *et al.* The p53 target Wig-1 regulates p53 mRNA stability through an AU-rich element. *Proc Natl Acad Sci U S A* 2009; **106**: 15756–61.

110 Kim BC, Lee HC, Lee J-J, Choi C-M, Kim D-K, Lee JC *et al.* Wig1 prevents cellular senescence by regulating p21 mRNA decay through control of RISC recruitment. EMBO J. 2012.

111 Chen DB, Yang HJ. Comparison of gene regulatory networks of benign and malignant breast cancer samples with normal samples. 2014; **13**: 9453–9462.

112 Apostolidis P a., Lindsey S, Miller WM, Papoutsakis ET. Proposed megakaryocytic regulon of p53: the genes engaged to control cell cycle and apoptosis during megakaryocytic differentiation. *Physiol Genomics* 2012; **44**: 638–650.

113 Parikh N, Hilsenbeck S, Creighton CJ, Dayaram T, Shuck R, Shinbrot E *et al.* Effects of TP53 mutational status on gene expression patterns across 10 human cancer types. *J Pathol* 2014; **232**: 522–533.

114 Wilhelm MT, Méndez-Vidal C, Wiman KG. Identification of functional p53-binding motifs in the mouse wig-1 promoter. *FEBS Lett* 2002; **524**: 69–72.

115 Zambelli F, Prazzoli GM, Pesole G, Pavesi G. Cscan: Finding common regulators of a set of genes by using a collection of genome-wide ChIP-seq datasets. *Nucleic Acids Res* 2012; **40**: 510–515.

116 Seo J, Lee K-J. Post-translational modifications and their biological functions: proteomic analysis and systematic approaches. *J Biochem Mol Biol* 2004; **37**: 35–44.

117 Jensen ON. Modification-specific proteomics: systematic strategies for analysing post-translationally modified proteins. *Trends Biotechnol* 2000; **18**: 36–42.

118 Sefton BM. Analysis of Protein Phosphorylation. *Curr Protoc Mol Biol* 2007.

119 Yu T, Wang P-Y, Rao JN, Zou T, Liu L, Xiao L *et al.* Chk2-dependent HuR phosphorylation regulates occludin mRNA translation and epithelial barrier function. *Nucleic Acids Res* 2011; **39**: 8472–87.

120 Clement SL, Scheckel C, Stoecklin G, Lykke-Andersen J. Phosphorylation of Tristetraprolin by MK2 Impairs AU-Rich Element mRNA Decay by Preventing Deadenylase Recruitment. *Mol Cell Biol* 2011; **31**: 256–266.

121 Doller A, Schlepckow K, Schwalbe H, Pfeilschifter J, Eberhardt W. Tandem phosphorylation of serines 221 and 318 by protein kinase Cdelta coordinates mRNA binding and nucleocytoplasmic shuttling of HuR. *Mol Cell Biol* 2010; **30**: 1397–1410.

122 Sandler H, Stoecklin G. Control of mRNA decay by phosphorylation of tristetraprolin. *Biochem Soc Trans* 2008; **36**: 491–496.

123 Hyeon HK, Abdelmohsen K, Lal A, Pullmann R, Yang X, Galban S *et al.* Nuclear HuR accumulation through phosphorylation by Cdk1. *Genes Dev* 2008; **22**: 1804–1815.

124 Abdelmohsen K, Pullmann R, Lal A, Kim HH, Galban S, Yang X *et al.* Phosphorylation of HuR by Chk2 Regulates SIRT1 Expression. *Mol Cell* 2007; **25**: 543–557.

125 Monni O, Oinonen R, Elonen E, Franssila K, Teerenhovi L, Joensuu H *et al.* Gain of 3q and deletion of 11q22 are frequent aberrations in mantle cell lymphoma. *Genes Chromosom Cancer* 1998; **21**: 298–307.

126 Björkqvist AM, Husgafvel-Pursiainen K, Anttila S, Karjalainen A, Tammilehto L, Mattson K *et al.* DNA gains in 3q occur frequently in squamous cell carcinoma of the lung, but not in adenocarcinoma. *Genes Chromosom Cancer* 1998; **22**: 79–82.

127 Brass N, Râcz A, Heckel D, Remberger K, Sybrecht GW, Meese EU. Amplification of the genes BCHE and SLC2A2 in 40% of squamous cell carcinoma of the lung. *Cancer Res* 1997; **57**: 2290–2294.

128 Sattler HP, Lensch R, Rohde V, Zimmer E, Meese E, Bonkhoff H *et al.* Novel amplification unit at chromosome 3q25-q27 in human prostate cancer. *Prostate* 2000; **45**: 207–215.

129 Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893–2917.

130 Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang X-S *et al.* Global surveillance of cancer survival 1995–2009: analysis of individual data for 25 676 887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet* 2014; **2025**: 1–34.

131 Andrae B, Kemetli L, Sparén P, Silfverdal L, Strander B, Ryd W *et al.* Screening-preventable cervical cancer risks: Evidence from a nationwide audit in Sweden. *J Natl Cancer Inst* 2008; **100**: 622–629.

132 Vaccarella S, Lortet-Tieulent J, Plummer M, Franceschi S, Bray F. Worldwide trends in cervical cancer incidence: Impact of screening against changes in disease risk factors. *Eur J Cancer* 2013; **49**: 3262–3273.

133 Vaccarella S, Franceschi S, Engholm G, Lönnberg S, Khan S, Bray F. 50 years of screening in the Nordic countries: quantifying the effects on cervical cancer incidence. *Br J Cancer* 2014; : 1–5.

134 Dillner J. Cervical cancer screening in Sweden. *Eur J Cancer* 2000; **36**: 2255–9.

135 Crow JM. HPV : THE GLOBAL BURDEN. *Nature* 2012.

136 Ferlay J. Cancer Incidence in Five Continents , Volumes I to VIII. *Cancer* 2005.

137 Berrington de González a, Sweetland S, Green J. Comparison of risk factors for squamous cell and adenocarcinomas of the cervix: a meta-analysis. *Br J Cancer* 2004; **90**: 1787–1791.

138 Andersson S, Rylander E, Larsson B, Strand a, Silfversvärd C, Wilander E. The role of human papillomavirus in cervical adenocarcinoma carcinogenesis. *Eur J Cancer* 2001; **37**: 246–50.

139 Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. *J Infect Dis* 2007; **195**: 1582–1589.

140 Huibregtse JM, Scheffner M, Howley PM. A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. *EMBO J* 1991; **10**: 4129–4135.

141 Thomas M, Pim D, Banks L. The role of the E6-p53 interaction in the molecular pathogenesis of HPV. *Oncogene* 1999; **18**: 7690–7700.

142 Scheffner M, Münger K, Byrne JC, Howley PM. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. *Proc Natl Acad Sci U S A* 1991; **88**: 5523–5527.

143 Scheffner M, Münger K, Huibregtse JM, Howley PM. Targeted degradation of the retinoblastoma protein by human papillomavirus E7-E6 fusion proteins. *EMBO J* 1992; **11**: 2425–2431.

144 Wright TC, Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. *N Engl J Med* 2003; **348**: 489–490.

145 Costa S. Performance of HPV DNA testing in the follow-up after treatment of high-grade cervical lesions, adenocarcinoma in situ (AIS) and microinvasive carcinoma. *Ecancermedicalscience* 2015; **9**: 1–7.

146 Burger MP, Hollema H, Pieters WJ, Schroder FP, Quint WG. Epidemiological evidence of cervical intraepithelial neoplasia without the presence of human papillomavirus. *BrJ Cancer* 1996; **73**: 831–836.

147 Riou G, Favre M, Jeannel D, Bourhis J, Le Doussal V, Orth G. Association between poor prognosis in early-stage invasive cervical carcinomas and non-detection of HPV DNA. *Lancet* 1990; **335**: 1171–1174.

148 Higgins GD, Davy MR, Roder D, Uzelin DM, Phillips GE, Burrell CJ. Increased age and mortality associated with cervical carcinomas negative for human papillomavirus RNA. *Lancet* 1991; **338**: 910–913.

149 Rodríguez-Carunchio L, Soveral I, Steenbergen R, Torné a, Martinez S, Fusté P *et al.* HPV-negative carcinoma of the uterine cervix: a distinct type of cervical cancer with poor prognosis. *BJOG An Int J Obstet Gynaecol* 2015; **122**: 119–127.

150 Park DJ, Wilczynski SP, Paquette RL, Miller CW, Koeffler HP. p53 mutations in HPV-negative cervical carcinoma. *Oncogene* 1994; **9**: 205–210.

151 Zhang A, Månér S, Betz R, Ångström T, Stendahl U, Bergman F *et al.* Genetic alterations in cervical carcinomas: Frequent low-level amplifications of oncogenes are associated with human papillomavirus infection. *Int J Cancer* 2002; **101**: 427–433.

152 Heselmeyer K, Schröck E, du Manoir S, Blegen H, Shah K, Steinbeck R *et al.* Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. *Proc Natl Acad Sci U S A* 1996; **93**: 479–484.

153 Heselmeyer-Haddad K, Sommerfeld K, White NM, Chaudhri N, Morrison LE, Palanisamy N *et al.* Genomic amplification of the human telomerase gene (TERC) in pap smears predicts the development of cervical cancer. *Am J Pathol* 2005; **166**: 1229–1238.

154 Andersson S, Wallin K-L, Hellström A-C, Morrison LE, Hjerpe A, Auer G *et al.* Frequent gain of the human telomerase gene TERC at 3q26 in cervical adenocarcinomas. *Br J Cancer* 2006; **95**: 331–338.

155 Gilbert SF. Developmental Biology -- NCBI Bookshelf. Dev. Biol. 2000.

156 Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A* 1981; **78**: 7634–7638.

157 Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981; **292**: 154–156.

158 Keirstead HS, Nistor G, Bernal G, Totoiu M, Cloutier F, Sharp K *et al.* Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants

remyelinate and restore locomotion after spinal cord injury. *J Neurosci* 2005; **25**: 4694–705.

159 Tadros W, Lipshitz HD. The maternal-to-zygotic transition: a play in two acts. *Development* 2009; **136**: 3033–3042.

160 Pikó L, Clegg KB. Quantitative changes in total RNA, total poly(A), and ribosomes in early mouse embryos. *Dev Biol* 1982; **89**: 362–378.

161 Okabe M. The cell biology of mammalian fertilization. *Development* 2013; **140**: 4471–9.

162 Schier AF. The maternal-zygotic transition: death and birth of RNAs. *Science* 2007; **316**: 406–7.

163 Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 1988; **332**: 459–461.

164 Deng Q, Ramsköld D, Reinius B, Sandberg R. Single-cell RNA-seq reveals dynamic, random monoallelic gene expression in mammalian cells. *Science* 2014; **343**: 193–6.

165 Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton D a. 'Stemness': transcriptional profiling of embryonic and adult stem cells. *Science* 2002; **298**: 597–600.

166 Park I, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL *et al.* Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* 2003; **423**: 302–305.

167 Chia N-Y, Chan Y-S, Feng B, Lu X, Orlov YL, Moreau D *et al.* A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. *Nature* 2010; **468**: 316–20.

168 Liu Z, Scannell DR, Eisen MB, Tjian R. Control of embryonic stem cell lineage commitment by core promoter factor, TAF3. *Cell* 2011; **146**: 720–731.

169 Kwon SC, Yi H, Eichelbaum K, Föhr S, Fischer B, You KT *et al.* The RNA-binding protein repertoire of embryonic stem cells. *Nat Struct Mol Biol* 2013; **20**: 1122–30.

170 Sheiness D, Fanshier L, Bishop JM. Identification of nucleotide sequences which may encode the oncogenic capacity of avian retrovirus MC29. *J Virol* 1978; **28**: 600–610.

171 Mellon P, Pawson A, Bister K, Martin GS, Duesberg PH. Specific RNA sequences and gene products of MC29 avian acute leukemia virus. *Proc Natl Acad Sci U S A* 1978; **75**: 5874–5878.

172 Vennstrom B, Sheiness D, Zabielski J, Bishop JM. Isolation and characterization of c-myc, a cellular homolog of the oncogene (v-myc) of avian myelocytomatosis virus strain 29. *J Virol* 1982; **42**: 773–779.

173 Manfred S, Kari A, Karl-Heinz K, E.Varmus H, Bishop JM, Gilbert F *et al.* Amplified DNA with limited homology to myc cellular oncogene is shared by human neubleastoma cell lines and a neuroblastoma tumour. *Nature* 1983; **305**: 245–248.

174 Kohl NE, Kanda N, Schreck RR, Bruns G, Latt SA, Gilbert F *et al.* Transposition and amplification of oncogene-related sequences in human neuroblastomas. *Cell* 1983; **35**: 359–367.

175 Nau MM, Brooks BJ, Battey J, Sausville E, Gazdar a F, Kirsch IR *et al.* L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. *Nature* 1985; **318**: 69–73.

176 Vita M, Henriksson M. The Myc oncoprotein as a therapeutic target for human cancer. Semin. Cancer Biol. 2006; **16**: 318–330.

177 Nesbit CE, Tersak JM, Prochownik E V. MYC oncogenes and human neoplastic disease. *Oncogene* 1999; **18**: 3004–3016.

178 Schwab M. MYCN in neuronal tumours. Cancer Lett. 2004; **204**: 179–187.

179 Blackwell TK, Kretzner L, Blackwood EM, Eisenman RN, Weintraub H. Sequence-specific DNA binding by the c-Myc protein. *Science* 1990; **250**: 1149–1151.

180 Tansey WP. Mammalian MYC Proteins and Cancer. *New J Sci* 2014; **2014**: 1–27.

181 Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. *Nat Rev Cancer* 2002; **2**: 764–776.

182 Kress TR, Sabò A, Amati B. MYC: connecting selective transcriptional control to global RNA production. *Nat Rev Cancer* 2015; **15**: 593–607.

183 Rounbehler RJ, Fallahi M, Yang C, Steeves MA, Li W, Doherty JR *et al.* Tristetraprolin impairs Myc-induced lymphoma and abolishes the malignant state. *Cell* 2012; **150**: 563–574.

184 Domashenko AD, Latham KE, Hatton KS. Expression of myc-family, myc-interacting, and myc-target genes during preimplantation mouse development. *Mol Reprod Dev* 1997; **47**: 57–65.

185 Stanton BR, Perkins AS, Tessarollo L, Sassoon DA, Parada LF. Loss of N-myc function results in embryonic lethality and failure of the epithelial component of the embryo to develop. *Genes Dev* 1992; **6**: 2235–2247.

186 Sawai S, Shimono A, Hanaoka K, Kondoh H. Embryonic lethality resulting from disruption of both N-myc alleles in mouse zygotes. *Genes Dev* 1992; **6**: 861–869.

187 Moens CB, Stanton BR, Parada LF, Rossant J. Defects in heart and lung development in compound heterozygotes for two different targeted mutations at the N-myc locus. *Development* 1993; **119**: 485–499.

188 Charron J, Malynn B a., Fisher P, Stewart V, Jeannette L, Goff SP *et al.* Embryonic lethality in mice homozygous for a targeted disruption of the N-myc gene. *Genes Dev* 1992; **6**: 2248–2257.

189 Davis AC, Wims M, Spotts GD, Hann SR, Bradley A. A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. *Genes Dev* 1993; **7**: 671–682.

190 Moens CB, Auerbach AB, Conlon RA, Joyner AL, Rossant J. A targeted mutation reveals a role for N-myc in branching morphogenesis in the embryonic mouse lung. *Genes Dev* 1992; **6**: 691–704.

191 Sawai S, Shimono a, Wakamatsu Y, Palmes C, Hanaoka K, Kondoh H. Defects of embryonic organogenesis resulting from targeted disruption of the N-myc gene in the mouse. *Development* 1993; **117**: 1445–1455.

192 Hatton KS, Mahon K, Chin L, Chiu FC, Lee HW, Peng D *et al.* Expression and activity of L-Myc in normal mouse development. *Mol Cell Biol* 1996; **16**: 1794–1804.

193 Grandori C, Cowley SM, James LP, Eisenman RN. T He Myc / Max / Mad Network and the transcriptional control of cell behavior. *Annu DevBiol* 2000; **16**: 653–699.

194 Nagy a, Moens C, Ivanyi E, Pawling J, Gertsenstein M, Hadjantonakis a K *et al.* Dissecting the role of N-myc in development using a single targeting vector to generate a series of alleles. *Curr Biol* 1998; **8**: 661–664.

195 Shen-Li H, O'Hagan RC, Hou H, Horner JW, Lee HW, DePinho R a. Essential role for Max in early embryonic growth and development. *Genes Dev* 2000; **14**: 17–22.

196 Malynn B a, Alboran IM De, Hagan RCO, Hagan CO, Bronson R, Davidson L *et al.* N- myc can functionally replace c- myc in murine development , cellular growth , and differentiation. 2000; : 1390–1399.

197 Cartwright P. LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. *Development* 2005; **132**: 885–896.

198 Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007; **448**: 313–317.

199 Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* 2006; **126**: 663–676.

200 Rodolfa KT, Eggan K. A Transcriptional Logic for Nuclear Reprogramming. *Cell* 2006; **126**: 652–655.

201 Yamanaka S. Elite and stochastic models for induced pluripotent stem cell generation. *Nature* 2009; **460**: 49–52.

202 Varlakhanova N V, Cotterman RF, deVries WN, Morgan J, Donahue LR, Murray S *et al.* Myc Maintains Embryonic Stem Cell Pluripotency and Self-Renewal. *Differentiation* 2010; **80**: 9–19.

203 Ross R a, Lazarova DL, Manley GT, Smitt PS, Spengler B a, Posner JB *et al.* HuD , a Neuronal-Specific RNA-Binding Protein , is a Potential Regulator of MYCN Expression in Human Neuroblastoma Cells. *Science (80-)* 1997; **33**: 2071–2074.

204 Lazarova DL, Spengler BA, Biedler JL, Ross RA. HuD, a neuronal-specific RNA-binding protein, is a putative regulator of N-myc pre-mRNA processing/stability in malignant human neuroblasts. *Oncogene* 1999; **18**: 2703–2710.

205 Hyeon HK, Kuwano Y, Srikantan S, Eun KL, Martindale JL, Gorospe M. HuR recruits let-7/RISC to repress c-Myc expression. *Genes Dev* 2009; **23**: 1743–1748.

206 Liao B, Hu Y, Brewer G. Competitive binding of AUF1 and TIAR to MYC mRNA controls its translation. *Nat Struct Mol Biol* 2007; **14**: 511–518.

207 Kim BC, Lee HC, Lee J, Choi C, Kim D, Figure S *et al.* Wig1 Prevents Cellular Senescence via Regulation of p21 mRNA Decay through Control of RISC Recruitment. *EMBO J* 2012.

208 Bersani C, Xu L-D, Vilborg a, Lui W-O, Wiman KG. Wig-1 regulates cell cycle arrest and cell death through the p53 targets FAS and 14-3-3σ. *Oncogene* 2014; : 1–11.

209 Stanton BR, Perkins AS, Tessarollo L, Sassoon DA, Parada LF. Loss of N-myc function results in embryonic lethality and failure of the epithelial component of the embryo to develop. *Genes Dev* 1992; **6**: 2235–2247.

210 Davis a. C, Wims M, Spotts GD, Hann SR, Bradley a. A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. *Genes Dev* 1993; **7**: 671–682.

211 Liang S, Guo R, Zhang Z, Liu D, Xu H, Xu Z *et al.* Upregulation of the eIF4E signaling pathway contributes to the progression of gastric cancer, and targeting eIF4E by perifosine inhibits cell growth. *Oncol Rep* 2013; **29**: 2422–30.

212 Mamane Y, Petroulakis E, Rong L, Yoshida K, Ler LW, Sonenberg N. eIF4E--from translation to transformation. *Oncogene* 2004; **23**: 3172–3179.

213 Topisirovic I, Siddiqui N, Orolicki S, Skrabanek L a, Tremblay M, Hoang T *et al.* Stability of eukaryotic translation initiation factor 4E mRNA is regulated by HuR, and this activity is dysregulated in cancer. *Mol Cell Biol* 2009; **29**: 1152–1162.

214 Helton R, Cui J, Scheel JR, Ellison J a, Ames C, Gibson C *et al.* Brain-specific knock-out of hypoxia-inducible factor-1alpha reduces rather than increases hypoxic-ischemic damage. *J Neurosci* 2005; **25**: 4099–4107.

215 Jiang L, Kon N, Li T, Wang S, Su T, Hibshoosh H *et al.* Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 2015. doi:10.1038/nature14344.

216 Luo S, Bai Y, Lan H. Influence of interference of WIG-1 on the multi-drug resistence in small cell lung cancer. *Chin J Oncol* 2014; **36**.

217 Wen Y, Gamazon ER, Bleibel W, Wing C, Mi S, McIlwee B *et al.* An eQTL-based method identified CTTN and Zmat3 as pemetrexed susceptibility markers. Hum. Mol. Genet. 2012; **21**: 1470–1480.