Chapter 2
The Inheritance of p53

Lukasz F. Grochola, Jorge Zeron-Medina, Emmanouela Repapi, Alexander E. Finlayson, Ying Cai, Gurinder Singh Atwal, and Gareth L. Bond

1 Introduction

1.1 p53 in the Cell

Since its first description in 1979, the p53 protein and its cellular network of genes has constituted one of the most extensively studied areas in cancer biology (Lane and Levine 2010). In more than three decades of intense research, compelling evidence has established the role of the p53 pathway as a major cellular tumor suppressor network (Lane and Levine 2010). The p53 protein has been demonstrated to suppress cancer primarily through the induction of cell cycle arrest, apoptosis, or senescence in response to a wide range of different cellular stresses in a stimulus- and cell-type dependent manner (Lane and Levine 2010; Vousden and Prives 2009). The stress signals include genotoxic damage, hypoxia, loss of normal cell contact, nutrient deprivation, mitotic spindle damage, heat or cold shock, telomere shortening, unfolded proteins, and oncogene activation (Vousden and Prives 2009; Lane and Levine 2010). Upon cellular stress, the p53 protein undergoes a plethora of post-translational modifications, which can affect the stability and activity of the protein (Vousden and Prives 2009; Lane and Levine 2010). To elicit the cellular stress responses p53 primarily acts as a sequence-specific transcription factor, but it can also directly regulate proteins from the Bcl-2 family (Moll et al. 2005), microRNA processing (Suzuki et al. 2009), and lincRNA expression (Huarte et al. 2010).

The complex regulation of p53 enables it to integrate a variety of stress signals into specific cellular responses in a stimulus- and cell-type dependent manner

L.F. Grochola • J. Zeron-Medina • E. Repapi • A.E. Finlayson • G.L. Bond (✉)
The Ludwig Institute for Cancer Research, ORCRB, University of Oxford, Old Road Campus, Off Roosevelt Drive, Oxford OX3 7DQ, UK
e-mail: gareth.bond@ndm.ox.ac.uk

Y. Cai • G.S. Atwal
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, NY, USA

P. Hainaut et al. (eds.), *p53 in the Clinics*, DOI 10.1007/978-1-4614-3676-8_2,

Lane and Levine 2010; Lu 2010. An interesting component of this regulation is a multitude of feedback loops, which positively or negatively affect the activity of p53 (Lu 2010). Hereby, the MDM2-p53 feedback loop is thought to be the key regulatory component of the p53 stress response (Lu 2010). It controls the level of expression and the activity of p53 by different mechanisms (Lu 2010; Manfredi 2010). Murine double minute 2 (Mdm2) is an E3 ubiquitin ligase, which can bind the p53 protein and target it for proteosome-mediated degradation through ubiquitination (Manfredi 2010; Lu 2010). Furthermore, Mdm2 directly binds to the N-terminus of p53, thereby masking its transactivation domain (Manfredi 2010). In addition, Mdm2 has been demonstrated to shuttle p53 out of the nucleus, where it can no longer function as a transcriptional activator (Wade et al. 2010). Interestingly, *MDM2* itself is a transcriptional target of p53, thus resulting in a negative feedback loop between p53 and Mdm2 (Manfredi 2010; Lu 2010).

1.2 p53 in the Organism: Cancer

The impairment of this tightly regulated network of genes centered on p53 is a hallmark of tumorigenesis (Lane and Levine 2010; Lu 2010). In fact, it has been estimated that 50 % of human cancers carry somatic mutations of the *TP53* gene, which inactivate the normal function of p53 (Lane and Levine 2010; Olivier et al. 2010). In addition, human tumors that retain a *TP53* wild-type gene frequently show a partial abrogation of downstream effectors (e.g., *BAX*, *BAK*, and *APAF-1*) or upstream (e.g., *ATM*, *CHK2*, *MDM2*, and *p19ARF*) regulators of this network (Lane and Levine 2010; Wade et al. 2010).

Genes that constitute the p53 network do not only affect the susceptibility to cancer, but are also crucial in the mediation of the cellular response to treatment with DNA-damaging therapeutic agents. Those agents, such as radio- and chemotherapy, lead to the activation of the upstream effectors of p53 (e.g., Atm, Atr, Chk1, Chk2) (Zhou and Elledge 2000; Bolderson et al. 2009) that activate p53 and can ultimately lead to cancer cell clearance via activation of apoptotic or senescence pathways (Johnstone et al. 2002; Lowe and Lin 2000). Indeed, mutations in the *TP53* gene can produce multidrug resistance in cells and in mice, and reintroduction of wild-type p53 can reconstitute chemosensitivity (Johnstone et al. 2002; Wallace-Brodeur and Lowe 1999). By the same token, mutations or altered expression of other p53 network genes, such as *APAF-1* or *BCL-2*, can significantly alter drug sensitivity in experimental models and are associated with multidrug resistance in human cancers (Johnstone et al. 2002; Reed 1999; Schmitt et al. 2000a; Wei et al. 2001; Zhang et al. 2000).

1.3 p53 in the Organism: Reproduction

Besides its crucial role in maintaining genomic stability and preventing tumor formation, a growing body of evidence suggests that p53 also regulates reproductive processes and fecundity (Hu et al. 2008). *TP53* is conserved from invertebrates to

mammals (Belyi et al. 2010) and homologs of *TP53* have been described in many different organisms, such as sea anemone, clams, *Caenorhabditis elegans*, Drosophila, frogs, and zebra fish (Hu 2009; Hu et al. 2008). Interestingly, one of the primary functions of the *TP53* ancestral gene in worms and flies, which are short-lived, cancer-free organisms, seems to be to ensure germline genomic integrity and the fidelity of the developmental process by regulating germ cell replication in order to eliminate defective offspring from the population (Hu et al. 2008; Hu 2009). These primordial p53 activities in the surveillance of germ cell replication and fecundity are also retained in higher organisms (Hu et al. 2008; Hu 2009). For example, in mice and rats, p53 levels are very high during spermatogenesis and mice with reduced levels of p53 show germ cell degeneration during the meiotic prophase (Hu et al. 2008; Hu 2009). Furthermore, p53 has also been shown to regulate reproduction in mice, whereby loss of p53 causes a significant decrease in fertility particularly in female animals (Hu et al. 2007a). Recently, Hu et al. have shown that one crucial mechanism by which p53 regulates maternal reproduction in mice is through its target gene leukemia inhibitory factor (*LIF*), a multifunctional cytokine, which plays a crucial role in blastocyst implantation in a p53-dependent manner (Hu et al. 2007a). Taken together, the observations made in invertebrates and mammals suggest that, besides its important role in maintaining genomic stability in somatic cells, p53 is also responsible to ensure faithful development, germline genomic integrity and fertility, and thus the production of normal offspring that will survive and reproduce.

2 Low-Frequency, Highly Penetrant Inherited Mutations in Humans

2.1 Introduction

As mentioned earlier, evidence from over 30 years of intense research efforts has established that the p53 network of genes is crucial in suppressing cancer in vertebrates. In fact, it has been well documented that low-frequency, highly penetrant inherited mutations in genes that are either directly in the p53 signaling pathway, or in closely interacting pathways, underlie many known cancer predisposition syndromes. These include Ataxia telangiectasia (caused by a homozygous mutation in the *ATM* gene) (McKinnon 2004), Cowden disease, Lhermitte–Duclos disease, and Bannayan–Zonana syndrome (all caused by mutations in the *PTEN* gene) (Liaw et al. 1997; Marsh et al. 1997; Di Cristofano et al. 1998), Xeroderma pigmentosum (caused by mutations in the *XP* genes) (Kraemer et al. 1994), Tuberous sclerosis complex 1 and 2 (caused by disruptions in the *TSC1* and *TSC2* genes) (Miyoshi et al. 2002; Orlova and Crino 2010), or the Peutz–Jeghers Syndrome (caused by disruptions in the *LKB1* gene) (Miyoshi et al. 2002).

Many of the same somatic mutations of the *TP53* gene that are found at such high frequencies in cancer cells can also be found as inherited mutations in individuals of

the Li–Fraumeni cancer syndrome (LFS) (Varley 2003; Varley et al. 1997). This autosomal-dominant inherited cancer predisposition disorder with an estimated prevalence between 1:5,000 and 1:20,000 (0.02–0.005 %) in the United Kingdom and the U.S.A (Lalloo et al. 2003; Gonzalez et al. 2009) is characterized by a familial clustering of early onset tumors including osteosarcomas, soft-tissue sarcomas, breast cancers, leukemia, and brain tumors (Varley 2003; Varley et al. 1997). The causative role of mutant p53 in this syndrome has been supported by many mouse models. Mice that harbor germline mutations or deletions of the *TP53* gene display cancer phenotypes that closely resemble the human syndrome (Donehower and Lozano 2009; Lozano 2010). Specifically, animals with a germline deletion of one *TP53* allele ($p53^{+/-}$) or carrying hot-spot mutations in the DNA-binding domain of p53 (corresponding to the human R175H and R273H loci) develop a broad spectrum of tumors closely resembling LFS, particularly lymphomas, osteosarcomas, soft-tissue sarcomas, and breast tumors significantly earlier than their wild-type counterparts (Donehower and Lozano 2009; Lozano 2010). Importantly, the median tumor incidence in the p53-targeting murine models is 18 months, and thus, for a mammal with a lifespan of 3 years, is similar to the human tumor onset in p53-mutation carriers, whereby the average cancer incidence is of 50 % by the age of 30 years (Donehower and Lozano 2009).

2.2 Genetic Testing

Genetic screening for inherited *TP53* mutations and close surveillance of carriers in order to improve patient prognosis has been a subject of great debate and is riddled with challenges (American College of Medical Genetics Board of Directors 1995; Li 1995; Committee on Bioethics 2001; Evans et al. 2010; Villani et al. 2011). Some of the challenges have been the wide range of tumors, the variability of the penetrance of the mutations, limited preventive options for most of the cancers, and ethical issues concerning newborn screening (American College of Medical Genetics Board of Directors 1995; Li 1995; Committee on Bioethics 2001; Evans et al. 2010; Villani et al. 2011; Achatz et al. 2009). However, females with a germline *TP53* mutation can be given the option of prophylactic mastectomy to reduce the risk of breast cancer (Hartmann et al. 1999; Thull and Vogel 2004; Schneider and Garber 1993). Furthermore, it has been recommended that radiation treatment in germline *TP53* mutation carriers should be assessed with scrutiny, as ionizing radiation has been shown to significantly increase the risk of second malignancies in this patient group (Varley 2003; Hisada et al. 1998; Limacher et al. 2001). However, a recent prospective observational study provides strong support for the utilization of genetic screening for inherited *TP53* mutations and subsequent presymptomatic surveillance of carriers in order to identify low-grade and premalignant cancers before they progress to a more malignant state and, therefore, improve outcome (Villani et al. 2011). In this study, asymptomatic *TP53* mutation carriers were surveyed using a protocol of noninvasive biochemical and imaging techniques. Of the 33 *TP53* mutation

carriers identified, 18 underwent surveillance. Remarkably, 3-year survival was 100 % in the surveillance group and only 21 % in the nonsurveillance group.

Indeed, mass newborn screening for a relatively prevalent germline p53 mutation has been adopted in the state of Parana, Brazil (Achatz et al. 2007, 2009). This p53 mutation, R337H, is at an unusually high frequency (1:300 individuals, 0.3 %) in the Brazilian population (Achatz et al. 2007, 2009). It associates with a 25 % cancer risk at the age of 30 years compared to the estimated 50 % by 30 years for the above-described p53 mutations. Although the genetic screening remains controversial due to the above-mentioned criticisms associated with genetic testing (Achatz et al. 2009), it is the first mass-implemented approach to utilize the inherited genetics of the p53 pathway to identify population groups at higher risk for cancer with the goal of early detection and improving the prognosis of cancer.

3 High Frequency, Lesser Penetrant Human Polymorphisms

3.1 Lessons from Mice

Interestingly, results from multiple mouse models suggest that less penetrant alleles of p53 network genes could also significantly alter p53 signaling and affect cancer onset and progression (Mendrysa et al. 2003, 2006; Alimonti et al. 2010). For example, haploinsufficiency for the murine double minute proteins Mdm2 or Mdm4 leads to increased p53 activity exhibited as increased sensitivity to DNA damage, decreased transformation potential, and tumor development in mice (Terzian et al. 2007). Moreover, mouse models designed to express a hypomorphic Mdm2 allele, which resulted in an approximately 30 % reduction of overall Mdm2 expression, had significantly increased p53 transcriptional activation and apoptotic activities (Mendrysa et al. 2003). Furthermore, the mice also had a significantly reduced formation of cancer compared to wild-type Mdm2 animals in an intestinal tumor model (Mendrysa et al. 2006). Similar observations have been recently made for the tumor suppressor PTEN, a key component of the PTEN/PI3K/Akt pathway implicated in the regulation of multiple biological processes such as apoptosis, metabolism, cell proliferation, and cell growth (Blanco-Aparicio et al. 2007). The PTEN/PI3K/Akt pathway is tightly integrated within the p53 network and has been shown to affect the activity of p53 via multiple mechanisms (Mayo and Donner 2001; Ogawara et al. 2002; Zhou et al. 2003; Freeman et al. 2003; Li et al. 2006; Zhou et al. 2001). In a recent paper, Alimonti et al. engineered a hypomorphic allele for murine PTEN that reduced PTEN expression by only 20 % (Alimonti et al. 2010). Interestingly, the authors demonstrate that animals expressing only 80 % of normal PTEN levels show an increased susceptibility to developing various types of cancer (Alimonti et al. 2010). Together, these mouse models suggest that less penetrant alleles of p53 network genes could also significantly alter p53 signaling and affect cancer onset. Indeed, extensive study of human high frequency genetic variants in the p53 network of genes supports this hypothesis.

3.2 High Frequency Genetic Variation

Human genetic variation, defined as the differences in DNA sequence within the genome of individuals, can be broadly classified into two different nucleotide composition classes, the single nucleotide variants (SNVs), which include single nucleotide polymorphisms (SNPs), point mutations, and single nucleotide insertions or deletions (indels), and the structural variants (Feuk et al. 2006; Frazer et al. 2009). One single nucleotide variant, the SNP, constitutes the most frequently studied form of high frequency human genetic variation (Feuk et al. 2006; Frazer et al. 2009). They can be distinguished from point mutations by their higher frequency in a population, whereby the minor allele frequency (MAF) of a SNP has to exceed the arbitrary cutoff of at least 1 % in any population (Feuk et al. 2006; Frazer et al. 2009). SNPs are usually bi-morphic, with two out of four possible nucleotide bases (i.e., Guanine, Cytosine, Adenine, Thymine) present at a particular allelic locus in a population (http://www.ncbi.nlm.nih.gov/snp). They arise from single historical mutational events in the human DNA, most frequently through physiological errors in DNA replication during cell division or alternatively induced by exogenous DNA-damaging agents (Ku et al. 2010; International HapMap Consortium 2003). The new mutation, or "allele," is termed the derived allele and, the former, the ancestral allele (International HapMap Consortium 2003).

Each allele is initially associated with a set of alleles of other SNPs that were present on the particular chromosomal background on which the SNP arose (International HapMap Consortium 2003; Kelley and Swanson 2008). The specific set of alleles observed on a single chromosome is called a haplotype (International HapMap Consortium 2003; Kelley and Swanson 2008). The coinheritance of SNP alleles in haplotypes results in associations between the respective alleles in a given population that is called linkage disequilibrium (LD) (International HapMap Consortium 2003; Neale 2010). Over time, chromosomal recombination during meiosis results in the reduction of length of each haplotype, and thus the LD between SNPs, whereby the probability of recombination between two SNPs increases with the distance between them (Slatkin 2008). The level of linkage disequilibrium is influenced by a number of factors, including the local rate of recombination, non-random mating, population subdivision and population bottlenecks, mutation, genetic drift, and natural selection (Slatkin 2008).

3.3 p53 Pathway High Frequency Genetic Variants in Mice and Humans

Two well-characterized SNPs in the p53 pathway are found in the *TP53* and *MDM2* genes (p53 codon72, rs1042522, C/G; MDM2 SNP309, rs2279744, T/G) and have been the subject of recent reviews (Vazquez et al. 2008; Grochola et al. 2010; Whibley et al. 2009). Both SNPs can be found at very high frequencies in certain

populations, but also vary significantly in their frequencies in different racial and ethnic groups. The p53 codon72 SNP can be as low as 33 % (in a Sub-Saharan African population, HapMap project) and as high as 77 % (in a Northern European population, dbSNP database). The MDM2 SNP309 can be as low as 10 % (in African Americans) and as high as 50 % (in Ashkenazi Jewish individuals) (Vazquez et al. 2008). Allele-specific natural selection has been proposed to explain theses differences and will be discussed below in more detail.

3.4 p53 Codon72 SNP

The p53 codon72 SNP encodes either a proline (p53-codon72-Pro) or arginine (p53-codon72-Arg) residue in a polyproline region of p53 that is located between the transactivation and the DNA-binding domains (Buchman et al. 1988) (Table 2.1). This polymorphism has been the subject of intense study and it is clear that the two different p53 variants differ in their activities significantly. For example, over 12 years ago, the first study to provide evidence that the two different p53 isoforms encoded by the p53 codon72 SNP are not functionally equivalent was published (Thomas et al. 1999). One of the differences the authors observed in cell culture experiments was that the codon72-Pro variant possesses increased ability to induce cell cycle arrest and that p53-codon72-Arg was more efficient at inducing apoptosis (Thomas et al. 1999). However, the precise molecular mechanisms by which these differences occur are still the subject of great debate in the literature (Vazquez et al. 2008; Grochola et al. 2010; Whibley et al. 2009).

Recent studies utilizing genetically engineered mice and mouse cells have shed light on the possible physiological effects of the p53 codon72 alleles (Frank et al. 2011; Zhu et al. 2010; Reinbold et al. 2008; Phang and Sabapathy 2007). The first major challenge these studies have had to overcome is the fact that the region surrounding codon 72 is not highly conserved in mice and encodes an alanine residue. Zhu et al. utilize mice with a humanized exon 4, which includes the sequence that encodes codon 72 (Arg/Pro). Frank et al. utilize mice with humanized exons 4 to 9, which they refer to as *hu*manized *p*53 *k*nock-*i*n gene (Hupki). Zhu et al. observe that their mice carrying the arginine residue associate with an increased rate of apoptosis in MEFs and in the small intestines after radiation compared to mice carrying the proline residue (Zhu et al. 2010). Frank et al. also observe that their mice carrying the arginine residue associate with an increased rate of apoptosis in those tissues after radiation compared to their mice carrying the proline residue (Frank et al. 2011). These data from both models are consistent with observations made in human tumor-derived cell culture systems (Grochola et al. 2010; Vazquez et al. 2008).

Frank et al. go on to show that the allelic differences in apoptotic potential for p53 codon72 are tissue specific and that in another tissue it is the proline isoform that associates with more apoptosis. Specifically, in thymocytes, p53 codon72-Pro mice have increased apoptosis following ionizing radiation along with an increased transactivation of a subset of p53 target genes (Frank et al. 2011). The authors

Table 2.1 Single nucleotide polymorphisms in the p53 pathway

Gene			Single nucleotide polymorphism			
Hugo name	Function	Association with the p53 pathway	Description	db SNP rs#ID	Clinical association	Molecular description
TP53	Tumor suppressor; functions as transcription factor, responds to diverse cellular stresses. Regulates target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism	Central node of the p53 pathway	p53 codon72	rs1042522	Many examples, such as allelic differences in response to chemotherapeutic treatment of head and neck carcinomas	Proline to arginine residue change in a region important in mediating the apoptotic response affects the level of apoptosis
MDM2	Key negative regulator of the p53 protein	Transcriptional target of p53; inhibits p53 activity by binding the transactivation domain of the p53 protein and promoting its ubiquitination as well as regulating its cellular location	MDM2 SNP309	rs2279744	Increased risk for and earlier age of onset of various cancer types, particularly in younger females	T to G change in the promoter region of intron 1 results in an enhanced binding affinity of the transcription factor SP1 and increased MDM2 gene transcription
			MDM2 SNP285	N.A.[a]	Modifier of MDM2 SNP309 phenotype	SNP285G>C diminishes Sp1 transcription factor binding to the MDM2 promoter

CASP8	An upstream protease of the activation cascade of caspases responsible for the death receptor-induced apoptosis	*TP53* activates caspase 8 gene expression after induction by death effector domains or exposure to cytotoxic drugs. Positive feedback loop with p53	D302H	rs1045485	Minor allele associates with reduced incidence of breast cancer	Aspartic acid to histidine substitution in codon 302
ATM	Serine/threonine protein kinase, which activates checkpoint signaling upon genotoxic stresses	Regulates the phosphorylation of p53 on various residues and activates MDM2 as well as the checkpoint kinase CHK2	ATM Ser49Cys	rs1800054	Minor allele suggested to associate with increased cancer risk, particularly breast carcinoma	Serine to cystein substitution in codon 49
CDKN1A	Binds and inhibits the activity of cyclin-dependent kinases, and thus functions as a regulator of cell cycle progression at G1	Tightly controlled by p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli	p21 codon31	rs1801270	Allelic differences reported for various cancer types	Serine to arginine substitution in a highly conserved region of the gene; alleles suggested to differ in transcriptional efficiency
CDKN1B	Cyclin-dependent kinase inhibitor; binds to and prevents the activation of cyclin E-CDK2 or cyclin D-CDK4 complexes, and thus controls the cell cycle at G1	Interacts with p53 to modulate the cell cycle and suppress tumorigenesis	–79C/T	rs34330	The T-allele associates with increased risk of breast cancer	C to T transition 5′ UTR at the site of nucleotide –79
TP73	Member of p53 family of transcription factors, involved in cellular responses to stress and development	TP73 transactivates p53-responsive genes causing cell cycle arrest and apoptosis; some isoforms can directly inhibit p53 function	G4C14-to-A4T14	rs2273953 rs1801173	Allelic differences for various cancer types	Two linked intronic SNPs, just upstream of the initiating AUG of exon 2 in position 4 and 14 of the gene

(continued)

Table 2.1 (continued)

Gene			Single nucleotide polymorphism			
Hugo name	Function	Association with the p53 pathway	Description	db SNP rs#ID	Clinical association	Molecular description
MDM4	Key negative regulator of the p53 protein	Transcriptional target of p53; inhibits p53 activity by binding the transactivation domain of the p53 protein. Heterodimerizes with the Mdm2 protein	Selected MDM4 haplotype	rs2369244; rs1563828	Selected haplotype associates with decreased risk and/or earlier age of breast and ovarian cancers	N.A.[a]
PPP2R5E	Regulatory subunit B, beta of protein phosphatase 2A	Major cellular phosphatase, regulates the phosphorylation of p53 and MDM2	epsilon-SNP2	rs11158491	Altered cancer risk and survival of soft-tissue sarcomas	N.A.[a]
PPP2R2B	Regulatory subunit B, beta of protein phosphatase 2A	Major cellular phosphatase, regulates the phosphorylation of p53 and MDM2	rs319217	rs319217	Altered breast cancer risk and recurrence, cellular chemosensitivities	N.A.[a]
TSC1	Encodes a growth inhibitory protein thought to play a role in the stabilization of tuberin	p53 regulation of mTOR activity depends on TSC1/2 complex formation	rs7874234	rs7874234	Allelic differences in age of ER-positive breast cancer tumor onset	T-allele of TSC1 SNP rs7874234 may create an estrogen receptor element (ERE) site

[a]*N.A.* not assigned

provide a possible explanation for the tissue specificity that is based on their observations that a group of genes induced by irradiation in the thymus are different than in other tissues, such as MEFs and the small intestines (Frank et al. 2011). This group is made up of genes that have known roles in immunity and inflammation (Frank et al. 2011). Interestingly, the authors go on to explore the possible differences in the p53 isoforms directly in the inflammatory response. They were able to demonstrate that upon inflammatory stimulation of the mice through endotoxin lipopolysaccharide, those mice harboring the proline residue are significantly more likely to succumb to septic shock than animals carrying the arginine residue (Frank et al. 2011).

Intriguingly, both Frank et al. and Zhu et al. were unable to find any differences between the p53 isoforms in cancer risk or outcome. Frank et al. crossed the proline and arginine mice with a mouse model that develops lymphomas (the Eμ-myc mouse), and with $p53^{+/-}$ mice that develop different cancers such as lymphomas and sarcomas (Donehower et al. 1992; Schmitt et al. 2000b; Frank et al. 2011). They observed no significant difference in the survival or tumor spectrum between the mice harboring the different alleles (Frank et al. 2011). Zhu et al. exposed their mice to chronic UVB treatment to induce skin cancer (Zhu et al. 2010). However, they also observed no statistically significant differences in susceptibility to skin cancer development in the animals (Zhu et al. 2010). Together, these data clearly demonstrate tissue-specific differences between the p53 proline and arginine isoforms in their abilities to induce apoptosis after DNA damage in vivo. Both models suggest that the p53 codon72 SNP alone might not have a direct impact on cancer development. However, a trend is emerging in association studies in human cancer patients that this SNP might work in conjunction with another functional p53 pathway SNP in the *MDM2* oncogene (MDM2 SNP309) to affect cancer risk (Wan et al. 2011).

3.5 MDM2 SNP309

MDM2 SNP309 is found at position 309 in the first intron of the *MDM2* oncogene, which serves as a transcriptional enhancer region (Bond et al. 2004) (Table 2.1). The SNP results in either a thymine (T) or a guanine (G) base. The G-allele increases the affinity of the transcription factor Sp1, which leads to the increased transcription and expression of Mdm2 and inhibition of the p53 stress response (Bond et al. 2004). Importantly, evidence from patient populations has also lent support to this model (Vazquez et al. 2008). For example, *TP53* mutation carriers (Li–Fraumeni individuals) with the G-allele of MDM2 SNP309 were shown to be diagnosed with tumors on average 7 years earlier than those that were T/T in genotype (Bond et al. 2004). This observation has been reproduced in three independent studies, in which *TP53* mutation carriers with the G-allele of MDM2 SNP309 were diagnosed with cancer on average 10, 16, and 12.5 years earlier than those who were homozygous for the T-allele (Bougeard et al. 2006; Ruijs et al. 2007; Marcel et al. 2009). Earlier ages of onset associated with individuals with the G-allele, but no known *TP53*

mutations, were also demonstrated in soft-tissue sarcomas, lymphoma, leukemia, head, neck, and oral squamous cell carcinomas, cancers of the colon, breast, bladder, ovary, brain, melanoma, and liver (Phillips et al. 2010; Whibley et al. 2009; Grochola et al. 2010).

To date, a plethora of case-control studies have explored the association of MDM2 SNP309 with overall cancer risk (Wan et al. 2011; Hu et al. 2007b). While many have shown a significant association of increased risk with the G-allele of SNP309 and various cancer types, such as lung, breast, and endometrial cancer, others have failed to provide support for these observations (Hu et al. 2007b; Wan et al. 2011; Ueda et al. 2009; Joshi et al. 2011; Fang et al. 2011). However, a recent meta-analysis of 66 papers that in total included over 25,000 cases and 30,000 controls comprising 30 tumor types provided evidence for an association of the G-allele of MDM2 SNP309 with increased overall cancer risk (Wan et al. 2011). The authors have shown that SNP309 G-allele carriers were associated with a significantly increased risk for various tumor types, such as breast, colorectal, and lung cancer, with a combined odds ratio of up to 1.25 (95 % confidence interval (CI) = 1.13–1.37), providing supportive evidence to the model that MDM2 SNP309 serves as a low-penetrance tumor susceptibility marker (Wan et al. 2011).

Similar to p53 codon72, a recent study evaluated the effects of MDM2 SNP309 on the p53 pathway and cancer risk by creating a mouse model (Post et al. 2010). The authors were motivated by being able to explore its impact on cancer risk in a system that excluded the genetic and environmental heterogeneity of the cancer phenotype inherent to human association studies. To do this, Post et al. generated mice that carry the humanized intron 1 of the *MDM2* gene, containing either the G- or the T-allele of MDM2 SNP309 (Post et al. 2010). Interestingly, the authors find that cells from animals with a G/G genotype of MDM2 SNP309 have elevated Mdm2 levels, reduced p53 levels, and decreased apoptosis. Moreover, and in contrast to the p53 codon72 mice, MDM2 SNP309 G/G mice have a shorter tumor latency and decreased survival, both in animals with two copies of wild-type p53 and in animals with one copy mutated ($p53^{515A/+}$) (Post et al. 2010). These data provide strong evidence that the G-allele of MDM2 SNP309 has a direct impact on cancer risk.

Interestingly, further studies in humans and human-derived material have demonstrated that the effects of the G-allele of MDM2 SNP309 on cancer can be modified by additional variables, such as gender, estrogen, and other p53 pathway SNPs (Bond et al. 2006a, b; Bond and Levine 2007; Lind et al. 2006; Alhopuro et al. 2005). Specifically, MDM2 SNP309 T/G has been repeatedly shown to associate with allele- and gender-specific differences in tumor diagnosis in various malignancies (Bartel et al. 2008; Bond et al. 2006a, b). Interestingly, female carriers of the G-allele have been shown to be diagnosed earlier in life with various cancers, such as colorectal cancer, diffuse large B-cell lymphoma, lung cancer, and for highly estrogen receptor positive (>50 % of tumor cells), but not for estrogen receptor negative, invasive ductal carcinoma of the breast (Bond et al. 2006a, b; Lind et al. 2006). This was shown to result in the enrichment of individuals with the G-allele in premenopausal women with these cancers, when compared to either

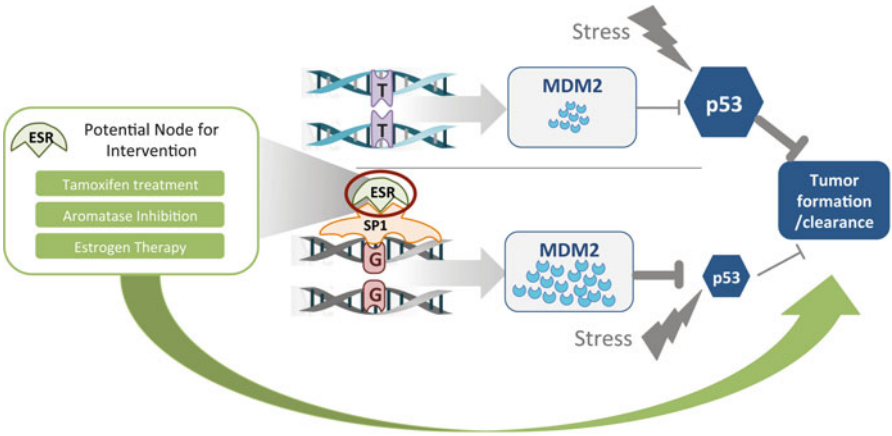

Fig. 2.1 The MDM2 SNP309 genotype could aid in the identification of individuals whose p53 pathway is attenuated in response to estrogen. MDM2 SNP309 is located in an enhancer region of *MDM2*. Multiple lines of evidence support a model that the G-allele has a higher affinity towards ER-alpha-Sp1-dependent activation of Mdm2 expression that leads to an attenuation of p53 activity. This model predicts that patients who carry a G-allele of MDM2 SNP309 would be more sensitive to estrogen signaling manipulation, such as Tamoxifen treatment or estrogen substitution, which is already being successfully used in both cancer prevention and treatment strategies

postmenopausal women or men with the same cancers. Subsequently, similar trends have been observed in melanoma and osteosarcoma patients (Firoz et al. 2009; Toffoli et al. 2009), while other studies suggest these trends will not be seen in every cancer and could be restricted to specific racial and ethnic backgrounds (Park et al. 2006; Bittenbring et al. 2008; Grochola et al. 2009). Molecular and functional studies suggest that the enhancer region of *MDM2* with the G-allele has a higher affinity towards ER-alpha-Sp1-dependent activation of Mdm2 expression (Grochola et al. 2010). This model requires further testing, however, as estrogen signaling manipulation is being successfully used in both cancer prevention and treatment strategies (Jordan 2006), the identification of a genetically defined population of women whose p53 pathway is attenuated in response to estrogen activation could positively affect clinical decisions (Fig. 2.1).

A recent report has provided evidence that a lower frequency SNP in the p53 network that resides in close proximity to SNP309 in intron 1 of the *MDM2* gene can affect the G-allele of MDM2 SNP309 in its ability to interact with Sp1 and affect cancer risk (Knappskog et al. 2011). Specifically, Knappskog et al. report that the C-allele of MDM2 SNP285 (G/C) is found only in Caucasians and with a minor allele frequency of 3.6–4.1 % compared to the 33.7–43.7 % frequency of MDM2 SNP309 (Table 2.1). They showed that the C-allele of MDM2 SNP285 strongly reduces the binding of Sp1 to the G-allele of MDM2 SNP309 in vitro (Knappskog et al. 2011). Moreover, the authors of the study show in a case-control study comprising 1,993 ovarian, 1,973 breast cancer patients, and 3,646 healthy controls that the C-allele of MDM2 SNP285 reduces the risk of both ovarian (OR 0.74;

CI 0.58–0.94) and breast cancers (OR 0.79; CI 0.62–1.00) among SNP309 G-allele carriers. Finally, in line with those results, they identified a population of women (MDM2 SNP309 G-allele and MDM2 SNP285 G-allele carriers) that had an increased risk for breast cancer (OR 1.27; CI 1.03–1.55) (Knappskog et al. 2011).

Evidence for a possible interaction between a higher frequency SNP in the p53 network is provided by another recent publication (Wan et al. 2011). Specifically, the above-mentioned meta-analysis of MDM2 SNP309 case-control studies by Wan et al., comprising 66 papers with over 25,000 cases and 30,000 controls suggests a significant interaction between MDM2 SNP309 and the p53 codon72 polymorphism (Wan et al. 2011). Hereby, the authors show that the combination of a SNP309G/G-codon72Pro/Pro genotype associated with an increased overall risk for cancer compared to the SNP309T/T-codon72Arg/Arg genotype (OR = 3.38, 95 % CI = 1.77–6.47), suggesting that there is an association between MDM2 SNP309 and p53 Arg72Pro regarding tumor susceptibility. Taken together, these reports suggest that in the future knowing the gender, estrogen exposures, and the genotypes of interacting SNP loci, such as MDM2 SNP309 and SNP285, or SNP309 and p53 codon72, could help to more precisely predict an individual's cancer risk and could influence future cancer therapies.

3.6 Haplotype Structures of p53 Pathway Genes and Natural Selection

Most SNPs are thought to arise due to mutations that do not influence the fitness of the organism and the relative proportion (frequency) of the alleles in the population changes over time by random genetic drift. Therefore, they may achieve significant frequencies in the human population simply by chance (Kelley and Swanson 2008; MacCallum and Hill 2006). However, some mutational events will give rise to true functional alleles. These changes in function could confer a fitness advantage to individuals causing the beneficial alleles to increase, on average, in frequency in the population (positive selection). However, should the changes in function be detrimental to the organism, the frequency of the mutation would decrease (negative selection) (Kelley and Swanson 2008; MacCallum and Hill 2006).

One consequence of the positive selection of an allele of a SNP and the subsequent selective sweep in a population is the increase in frequency of neutral alleles in other SNPs closely linked to the selected allele, an effect called the hitchhiking effect (Kelley and Swanson 2008; MacCallum and Hill 2006). This effect gives rise to hallmarks of allele-specific positive selection, namely, a reduction of nucleotide variation in the region of the genome surrounding the beneficial allele and a region characterized by a mix of long haplotypes harboring the selected allele and short haplotypes with the ancestral allele (Kelley and Swanson 2008; MacCallum and Hill 2006). Therefore, long high-frequency haplotypes indicate the action of positive selection, whereas nonselected haplotypes occur at lower frequencies and vary in length (Kelley and Swanson 2008; MacCallum and Hill 2006). In light of the

effect of natural selection on haplotype distributions, a haplotype-based statistical test was devised that detected evidence of natural selection on individual SNPs, and it was shown that SNP309 deviates significantly from the standard assumptions of models of selective neutrality (Atwal et al. 2007). The haplotype structure of *MDM2* was analyzed by combining genotype data generated from the International Hapmap Consortium, Celera Diagnostics, and Sheba Cancer Research Center in Israel (Atwal et al. 2007). A low level of haplotypic variability was observed in Northern European populations and Ashkenazi Jewish populations, reflecting strong linkage disequilibrium across the whole gene. The level of linkage disequilibrium was perceived to be stronger than that due to a population bottleneck effect, arising from a founder population out of Africa. The effect of a founder population bottleneck effect would reduce the genetic variability of the entire genome and not just a single gene. By comparisons with extensive computer simulations of molecular evolution it was suggested that the G-allele of MDM2 SNP309 had experienced a positive selection sweep in human populations. The authors reasoned that an evolutionary selection pressure could act on the human p53 pathway and a growing body of evidence now suggests that p53 regulates other human conditions that are under selective pressures, such as germline maintenance, fertility, and reproduction. Indeed, p53 codon72 polymorphism and MDM2 SNP309 have been shown to associate with recurrent implantation failure, missed abortion, and allelic differences in the outcome of in vitro fertilization treatment (Kang et al. 2009; Kay et al. 2006; Fang et al. 2009; Firouzabadi et al. 2009; Hu et al. 2007a; Atwal et al. 2007).

The finding that the G-allele of MDM2 SNP309 had experienced a positive selection sweep gave rise to the hypothesis that other functional SNPs in the p53 pathway could be determined by haplotype studies that looked for evidence of natural selection. Recent work has shown that using signatures of natural selection to identify putatively functional sites and variants has been a fruitful exercise in the study of human cancer. One of the first studies to demonstrate this was an investigation of the haplotype distribution of *MDM4* (Atwal et al. 2009), a structural homologue of *MDM2*, that binds to the amino terminus of p53, functioning as a major inhibitor of p53 activity (Wade et al. 2010). The haplotype structure was analyzed in several human populations. Genotype data for SNPs were collected from three sources: (1) a collection of 84 lymphoblastoid cell lines of African American and Caucasian ethnicity; (2) the Hapmap Project repository including the Caucasian (CEU) and African (YRI) populations; and (3) a cohort of 299 Ashkenazi Jewish controls from a breast cancer association study at Memorial Sloan Kettering Cancer Center. The low diversity of haplotypes from each dataset, resulting in atypical patterns of linkage disequilibrium, indicated the presence of candidate SNPs that may also modify the efficacy of the p53 pathway. In particular, one derived haplotype across the gene had increased to a high frequency (68 % in the Caucasian lymphoblastoid dataset, compared to 30 % in the African American population) in a relatively short amount of time in human history. Subsequent association studies in five different patient populations revealed that these SNPs in *MDM4* conferred an increased risk for, or early onset of, human breast and ovarian cancers in Ashkenazi Jewish and European cohorts, respectively (Atwal et al. 2009). This association was

subsequently validated in two patient cohorts, whereby the *MDM4* SNPs have been shown to associate with an accelerated age of onset of estrogen receptor negative breast cancer (Kulkarni et al. 2009) (Table 2.1). These observations not only suggest that, like *MDM2*, *MDM4* harbors SNPs that affect cancer in humans, but also that other SNPs in the p53 network that affect cancer could also be under selection pressures. Indeed multiple studies have lent support to this hypothesis and have identified candidate functional polymorphic loci in *PPP2R5E* (Grochola et al. 2009), *PPP2R2B* (Vazquez et al. 2011), *TSC1* (Mehta et al. 2011), *TSC2* (Mehta et al. 2011), and *HAUSP* (Kang et al. 2009) genes (Table 2.1).

With the onslaught of whole-genome sequences now being generated it will soon be possible to investigate the fine-detailed haplotype structure of the p53 pathway for large populations. The pilot phase of so-called The 1000 Genome Project has recently been completed with the aim of providing a deep characterization of the vast majority (over 95 %) of the common variations that are in genomic regions accessible to current high-throughput sequencing technologies and that have allele frequencies of 1 % or higher in a number of major population groups (The 1000 Genome Project Consortium). Genetic variations with an allele frequency as low as 0.1 % were also cataloged in coding regions since these regions tend to have reduced allele frequencies. This dataset furnishes us with the most fine-scale map to date of genotypes throughout the human genome across multiple different ethnicities. Preliminary haplotype studies on the dataset from the 1000 Genomes Project have illustrated that the *TP53* gene network is highly polymorphic, strengthening the hypothesis that there may be numerous subtle inherited genetic changes that impact the normal function of these different proteins (Cai and Atwal, unpublished). Despite the greater amount of newly discovered genetic variants in the *TP53* gene network, the genes appear to have slightly reduced levels of haplotypic diversity, indicating evidence of natural selection, corroborating the aforementioned earlier studies.

The above-described studies suggest that the p53 stress response pathway could harbor more functional inherited genetic variants. Indeed, other less studied polymorphic variants have been reported for the p53 network genes *CDKN1A*, *CDKN1B*, *TP73*, *ATM,* and *CASP8* (Grochola et al. 2010; Whibley et al. 2009) (Table 2.1). The identification of other functional SNPs that mediate the p53 stress response will prove challenging, as there are over 50,000 SNPs in the NCBI SNP repository (dbSNP) in genes that have been implicated in mediating and regulating the p53 response (Vazquez et al. 2008). However, recently, approaches have been described that could help identify potential functional p53 pathway SNPs (Smirnov et al. 2009; Tomso et al. 2005; Noureddine et al. 2009; Bandele et al. 2011; Grochola et al. 2010). Specifically, these strategies identify SNPs, or haplotypes, that demonstrate allelic differences in characteristics similar, but not limited to, functional p53 pathway SNPs, such as differences in cellular chemosensitivities and the ability to change p53-dependent transcriptional activation of genes. The more in-depth study of these and other genetic variants in the p53 network should help further define populations in their abilities to respond to stress, suppress tumor formation, and respond to DNA damaging therapies.

4 Conclusion

Taken together, mounting evidence suggests that the inherited genetics of p53 pathway have a great potential to further define populations in their abilities to react to stress, suppress tumor formation, and respond to therapies. Various cancer predisposition syndromes are caused by low-frequency, highly penetrant inherited mutations in the p53 network, the knowledge of which can be used to individually tailor cancer prevention and treatment strategies. In addition, it has been demonstrated that the p53 tumor suppressor pathway also harbors functional high-frequency, low-penetrance genetic variants that affect p53 signaling in cells, resulting in allelic differences in cancer risk, prognosis, and responses to chemotherapy. Observations such as these suggest that the insight gained from a thorough analysis of the functional human genetics of this important network of genes could offer novel cancer treatment and prevention strategies as well as personalize those that already exist.

References

Achatz MI, Hainaut P, Ashton-Prolla P (2009) Highly prevalent TP53 mutation predisposing to many cancers in the Brazilian population: a case for newborn screening? Lancet Oncol 10:920–925. doi:S1470-2045(09)70089-0 [pii] 10.1016/S1470-2045(09)70089-0

Achatz MI, Olivier M, Le Calvez F et al (2007) The TP53 mutation, R337H, is associated with Li-Fraumeni and Li-Fraumeni-like syndromes in Brazilian families. Cancer Lett 245:96–102. doi:S0304-3835(06)00005-X [pii] 10.1016/j.canlet.2005.12.039

Alhopuro P, Ylisaukko-Oja SK, Koskinen WJ et al (2005) The MDM2 promoter polymorphism SNP309T–>G and the risk of uterine leiomyosarcoma, colorectal cancer, and squamous cell carcinoma of the head and neck. J Med Genet 42:694–698

Alimonti A, Carracedo A, Clohessy JG et al (2010) Subtle variations in Pten dose determine cancer susceptibility. Nat Genet 42:454–458. doi:ng.556 [pii] 10.1038/ng.556

American College of Medical Genetics Board of Directors (1995) Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. American Society of Human Genetics Board of Directors, American College of Medical Genetics Board of Directors. Am J Hum Genet 57:1233–1241

Atwal GS, Bond GL, Metsuyanim S et al (2007) Haplotype structure and selection of the MDM2 oncogene in humans. Proc Natl Acad Sci USA 104:4524–4529

Atwal GS, Kirchhoff T, Bond EE et al (2009) Altered tumor formation and evolutionary selection of genetic variants in the human MDM4 oncogene. Proc Natl Acad Sci USA 106:10236–10241

Bandele OJ, Wang X, Campbell MR et al (2011) Human single-nucleotide polymorphisms alter p53 sequence-specific binding at gene regulatory elements. Nucleic Acids Res 39:178–189

Bartel F, Jung J, Bohnke A et al (2008) Both germ line and somatic genetics of the p53 pathway affect ovarian cancer incidence and survival. Clin Cancer Res 14:89–96

Belyi VA, Ak P, Markert E et al (2010) The origins and evolution of the p53 family of genes. Cold Spring Harb Perspect Biol 2:a001198. doi:cshperspect.a001198 [pii] 10.1101/cshperspect.a001198

Bittenbring J, Parisot F, Wabo A et al (2008) MDM2 gene SNP309 T/G and p53 gene SNP72 G/C do not influence diffuse large B-cell non-Hodgkin lymphoma onset or survival in central European Caucasians. BMC Cancer 8:116

Blanco-Aparicio C, Renner O, Leal JF et al (2007) PTEN, more than the AKT pathway. Carcinogenesis 28:1379–1386. doi:bgm052 [pii] 10.1093/carcin/bgm052

Bolderson E, Richard DJ, Zhou BB et al (2009) Recent advances in cancer therapy targeting proteins involved in DNA double-strand break repair. Clin Cancer Res 15:6314–6320. doi:1078-0432.CCR-09-0096 [pii] 10.1158/1078-0432.CCR-09-0096

Bond GL, Hirshfield KM, Kirchhoff T et al (2006a) MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. Cancer Res 66:5104–5110

Bond GL, Hu W, Bond EE et al (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119:591–602

Bond GL, Levine AJ (2007) A single nucleotide polymorphism in the p53 pathway interacts with gender, environmental stresses and tumor genetics to influence cancer in humans. Oncogene 26:1317–1323

Bond GL, Menin C, Bertorelle R et al (2006b) MDM2 SNP309 accelerates colorectal tumour formation in women. J Med Genet 43:950–952

Bougeard G, Baert-Desurmont S, Tournier I et al (2006) Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome. J Med Genet 43:531–533

Buchman VL, Chumakov PM, Ninkina NN et al (1988) A variation in the structure of the protein-coding region of the human p53 gene. Gene 70:245–252

Committee on Bioethics (2001) Ethical issues with genetic testing in pediatrics. Pediatrics 107:1451–1455

Di Cristofano A, Pesce B, Cordon-Cardo C et al (1998) Pten is essential for embryonic development and tumour suppression. Nat Genet 19:348–355

Donehower LA, Harvey M, Slagle BL et al (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356:215–221

Donehower LA, Lozano G (2009) 20 years studying p53 functions in genetically engineered mice. Nat Rev Cancer 9:831–841

Evans DG, Lunt P, Clancy T et al (2010) Childhood predictive genetic testing for Li-Fraumeni syndrome. Fam Cancer 9:65–69. doi:10.1007/s10689-009-9245-9

Fang F, Yu XJ, Yu L et al (2011) MDM2 309 T/G polymorphism is associated with colorectal cancer risk especially in Asians: a meta-analysis. Med Oncol 28(4):981–985

Fang Y, Kong B, Yang Q et al (2009) MDM2 309 polymorphism is associated with missed abortion. Hum Reprod 24:1346–1349

Feuk L, Carson AR, Scherer SW (2006) Structural variation in the human genome. Nat Rev Genet 7:85–97. doi:nrg1767 [pii] 10.1038/nrg1767

Firouzabadi RD, Ghasemi N, Rozbahani MA et al (2009) Association of p53 polymorphism with ICSI/IVF failure and recurrent pregnancy loss. Aust NZ J Obstet Gynaecol 49:216–219

Firoz EF, Warycha M, Zakrzewski J et al (2009) Association of MDM2 SNP309, age of onset, and gender in cutaneous melanoma. Clin Cancer Res 15:2573–2580

Frank AK, Leu JI, Zhou Y et al (2011) The codon 72 polymorphism of p53 regulates interaction with NF-{kappa}B and transactivation of genes involved in immunity and inflammation. Mol Cell Biol 31:1201–1213

Frazer KA, Murray SS, Schork NJ et al (2009) Human genetic variation and its contribution to complex traits. Nat Rev Genet 10:241–251. doi:nrg2554 [pii] 10.1038/nrg2554

Freeman DJ, Li AG, Wei G et al (2003) PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms. Cancer Cell 3:117–130

Gonzalez KD, Noltner KA, Buzin CH et al (2009) Beyond Li Fraumeni syndrome: clinical characteristics of families with p53 germline mutations. J Clin Oncol 27:1250–1256. doi:JCO.2008.16.6959 [pii] 10.1200/JCO.2008.16.6959

Grochola LF, Vazquez A, Bond EE et al (2009) Recent natural selection identifies a genetic variant in a regulatory subunit of protein phosphatase 2a that associates with altered cancer risk and survival. Clin Cancer Res 15(19):6301–6308

Grochola LF, Muller TH, Bond GL et al (2010) MDM2 SNP309 associates with accelerated pancreatic adenocarcinoma formation. Pancreas 39(1):76–80

Grochola LF, Zeron-Medina J, Meriaux S et al (2010) Single-nucleotide polymorphisms in the p53 signaling pathway. Cold Spring Harb Perspect Biol 2:a001032. doi:cshperspect.a001032 [pii] 10.1101/cshperspect.a001032

Hartmann LC, Schaid DJ, Woods JE et al (1999) Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med 340:77–84. doi:10.1056/NEJM199901143400201

Hisada M, Garber JE, Fung CY et al (1998) Multiple primary cancers in families with Li-Fraumeni syndrome. J Natl Cancer Inst 90:606–611

Hu W (2009) The role of p53 gene family in reproduction. Cold Spring Harb Perspect Biol 1:a001073. doi:cshperspect.a001073 [pii] 10.1101/cshperspect.a001073

Hu W, Feng Z, Atwal GS et al (2008) p53: a new player in reproduction. Cell Cycle 7:848–852

Hu W, Feng Z, Teresky AK et al (2007a) p53 regulates maternal reproduction through LIF. Nature 450:721–724

Hu Z, Jin G, Wang L et al (2007b) MDM2 promoter polymorphism SNP309 contributes to tumor susceptibility: evidence from 21 case-control studies. Cancer Epidemiol Biomarkers Prev 16:2717–2723

Huarte M, Guttman M, Feldser D et al (2010) A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 142:409–419. doi:S0092-8674(10)00730-0 [pii] 10.1016/j.cell.2010.06.040

International HapMap Consortium (2003) The International HapMap Project. Nature 426:789–796. doi:10.1038/nature02168 nature02168 [pii]

Johnstone RW, Ruefli AA, Lowe SW (2002) Apoptosis: a link between cancer genetics and chemotherapy. Cell 108:153–164

Jordan VC (2006) Tamoxifen (ICI46,474) as a targeted therapy to treat and prevent breast cancer. Br J Pharmacol 147(Suppl 1):S269–276. doi:0706399 [pii] 10.1038/sj.bjp. 0706399

Joshi AM, Budhathoki S, Ohnaka K et al (2011) TP53 R72P and MDM2 SNP309 polymorphisms and colorectal cancer risk: the Fukuoka colorectal cancer study. Jpn J Clin Oncol 41:232–238

Kang HJ, Feng Z, Sun Y et al (2009) Single-nucleotide polymorphisms in the p53 pathway regulate fertility in humans. Proc Natl Acad Sci USA 106:9761–9766

Kay C, Jeyendran RS, Coulam CB (2006) p53 tumour suppressor gene polymorphism is associated with recurrent implantation failure. Reprod Biomed Online 13:492–496

Kelley JL, Swanson WJ (2008) Positive selection in the human genome: from genome scans to biological significance. Annu Rev Genomics Hum Genet 9:143–160. doi:10.1146/annurev.genom.9.081307.164411

Knappskog S, Bjornslett M, Myklebust LM et al (2011) The MDM2 promoter SNP285C/309G haplotype diminishes Sp1 transcription factor binding and reduces risk for breast and ovarian cancer in Caucasians. Cancer Cell 19:273–282

Kraemer KH, Lee MM, Andrews AD et al (1994) The role of sunlight and DNA repair in melanoma and nonmelanoma skin cancer. The xeroderma pigmentosum paradigm. Arch Dermatol 130:1018–1021

Ku CS, Loy EY, Salim A et al (2010) The discovery of human genetic variations and their use as disease markers: past, present and future. J Hum Genet 55:403–415. doi:jhg201055 [pii] 10.1038/jhg.2010.55

Kulkarni DA, Vazquez A, Haffty BG et al (2009) A polymorphic variant in human MDM4 associates with accelerated age of onset of estrogen receptor negative breast cancer. Carcinogenesis 30:1910–1915

Lalloo F, Varley J, Ellis D et al (2003) Prediction of pathogenic mutations in patients with early-onset breast cancer by family history. Lancet 361:1101–1102. doi:S0140-6736(03)12856-5 [pii] 10.1016/S0140-6736(03)12856-5

Lane D, Levine A (2010) p53 Research: the past thirty years and the next thirty years. Cold Spring Harb Perspect Biol 2:a000893. doi:cshperspect.a000893 [pii] 10.1101/cshperspect.a000893

Li AG, Piluso LG, Cai X et al (2006) Mechanistic insights into maintenance of high p53 acetylation by PTEN. Mol Cell 23:575–587. doi:S1097-2765(06)00456-4 [pii] 10.1016/j.molcel.2006.06.028

Li FP (1995) Identification and management of inherited cancer susceptibility. Environ Health Perspect 103(Suppl 8):297–300
Liaw D, Marsh DJ, Li J et al (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 16:64–67
Limacher JM, Frebourg T, Natarajan-Ame S et al (2001) Two metachronous tumors in the radiotherapy fields of a patient with Li-Fraumeni syndrome. Int J Cancer 96:238–242. doi:10.1002/ijc.1021 [pii]
Lind H, Zienolddiny S, Ekstrom PO et al (2006) Association of a functional polymorphism in the promoter of the MDM2 gene with risk of nonsmall cell lung cancer. Int J Cancer 119:718–721
Lowe SW, Lin AW (2000) Apoptosis in cancer. Carcinogenesis 21(3):485–495
Lozano G (2010) Mouse models of p53 functions. Cold Spring Harb Perspect Biol 2:a001115. doi:cshperspect.a001115 [pii] 10.1101/cshperspect.a001115
Lu X (2010) Tied up in loops: positive and negative autoregulation of p53. Cold Spring Harb Perspect Biol 2:a000984. doi:cshperspect.a000984 [pii] 10.1101/cshperspect.a000984
MacCallum C, Hill E (2006) Being positive about selection. PLoS Biol 4:e87. doi:06-PLBI-ED-0101 [pii] 10.1371/journal.pbio.0040087
Manfredi JJ (2010) The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. Genes Dev 24:1580–1589. doi:24/15/1580 [pii] 10.1101/gad.1941710
Marcel V, Palmero EI, Falagan-Lotsch P et al (2009) TP53PIN3 and MDM2 SNP309 polymorphisms as genetic modifiers in the Li-Fraumeni syndrome: impact on age at first diagnosis. J Med Genet 46:766–772
Marsh DJ, Dahia PL, Zheng Z et al (1997) Germline mutations in PTEN are present in Bannayan-Zonana syndrome. Nat Genet 16:333–334
Mayo LD, Donner DB (2001) A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc Natl Acad Sci USA 98:11598–11603
McKinnon PJ (2004) ATM and ataxia telangiectasia. EMBO Rep 5:772–776. doi:10.1038/sj.embor.7400210 7400210 [pii]
Mehta MS, Vazquez A, Kulkarni DA et al (2011) Polymorphic variants in TSC1 and TSC2 and their association with breast cancer phenotypes. Breast Cancer Res Treat 125:861–868. doi:10.1007/s10549-010-1062-1
Mendrysa SM, McElwee MK, Michalowski J et al (2003) mdm2 Is critical for inhibition of p53 during lymphopoiesis and the response to ionizing irradiation. Mol Cell Biol 23:462–472
Mendrysa SM, O'Leary KA, McElwee MK et al (2006) Tumor suppression and normal aging in mice with constitutively high p53 activity. Genes Dev 20:16–21
Miyoshi H, Nakau M, Ishikawa TO et al (2002) Gastrointestinal hamartomatous polyposis in Lkb1 heterozygous knockout mice. Cancer Res 62:2261–2266
Moll UM, Wolff S, Speidel D et al (2005) Transcription-independent pro-apoptotic functions of p53. Curr Opin Cell Biol 17:631–636. doi:S0955-0674(05)00146-8 [pii] 10.1016/j.ceb.2005.09.007
Neale BM (2010) Introduction to linkage disequilibrium, the HapMap, and imputation. Cold Spring Harb Protoc 2010: pdb top74. doi:2010/3/pdb.top74[pii]10.1101/pdb.top74
Noureddine MA, Menendez D, Campbell MR et al (2009) Probing the functional impact of sequence variation on p53-DNA interactions using a novel microsphere assay for protein-DNA binding with human cell extracts. PLoS Genet 5:e1000462
Ogawara Y, Kishishita S, Obata T et al (2002) Akt enhances Mdm2-mediated ubiquitination and degradation of p53. J Biol Chem 277:21843–21850. doi:10.1074/jbc.M109745200 M109745200 [pii]
Olivier M, Hollstein M, Hainaut P (2010) TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol 2:a001008. doi:10.1101/cshperspect.a001008
Orlova KA, Crino PB (2010) The tuberous sclerosis complex. Ann NY Acad Sci 1184:87–105. doi:NYAS5117 [pii]10.1111/j.1749-6632.2009.05117.x

Park SH, Choi JE, Kim EJ et al (2006) MDM2 309T>G polymorphism and risk of lung cancer in a Korean population. Lung Cancer 54:19–24
Phang BH, Sabapathy K (2007) The codon 72 polymorphism-specific effects of human p53 are absent in mouse cells: implications on generation of mouse models. Oncogene 26:2964–2974
Phillips CL, Gerbing R, Alonzo T et al (2010) MDM2 polymorphism increases susceptibility to childhood acute myeloid leukemia: a report from the Children's Oncology Group. Pediatr Blood Cancer 55:248–253. doi:10.1002/pbc.22519
Post SM, Quintas-Cardama A, Pant V et al (2010) A high-frequency regulatory polymorphism in the p53 pathway accelerates tumor development. Cancer Cell 18:220–230
Reed JC (1999) Dysregulation of apoptosis in cancer. J Clin Oncol 17(9):2941–2953
Reinbold M, Luo JL, Nedelko T et al (2008) Common tumour p53 mutations in immortalized cells from Hupki mice heterozygous at codon 72. Oncogene 27:2788–2794
Ruijs MW, Schmidt MK, Nevanlinna H et al (2007) The single-nucleotide polymorphism 309 in the MDM2 gene contributes to the Li-Fraumeni syndrome and related phenotypes. Eur J Hum Genet 15:110–114
Schmitt CA, Rosenthal CT, Lowe SW (2000a) Genetic analysis of chemoresistance in primary murine lymphomas. Nat Med 6:1029–1035. doi:10.1038/79542
Schmitt CA, Wallace-Brodeur RR, Rosenthal CT et al (2000b) DNA damage responses and chemosensitivity in the E mu-myc mouse lymphoma model. Cold Spring Harb Symp Quant Biol 65:499–510
Schneider K, Garber J (1993) Li-Fraumeni Syndrome. doi:NBK1311 [bookaccession]
Slatkin M (2008) Linkage disequilibrium—understanding the evolutionary past and mapping the medical future. Nat Rev Genet 9:477–485. doi:nrg2361 [pii] 10.1038/nrg2361
Smirnov DA, Morley M, Shin E et al (2009) Genetic analysis of radiation-induced changes in human gene expression. Nature 459:587–591
Suzuki HI, Yamagata K, Sugimoto K et al (2009) Modulation of microRNA processing by p53. Nature 460:529–533. doi:nature08199 [pii] 10.1038/nature08199
Terzian T, Wang Y, Van Pelt CS et al (2007) Haploinsufficiency of Mdm2 and Mdm4 in tumorigenesis and development. Mol Cell Biol 27:5479–5485
Thomas M, Kalita A, Labrecque S et al (1999) Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 19:1092–1100
The 1000 Genomes Project Consortium, Nature (2010) 467, 1061–1073
Thull DL, Vogel VG (2004) Recognition and management of hereditary breast cancer syndromes. Oncologist 9:13–24
Toffoli G, Biason P, Russo A et al (2009) Effect of TP53 Arg72Pro and MDM2 SNP309 polymorphisms on the risk of high-grade osteosarcoma development and survival. Clin Cancer Res 15:3550–3556
Tomso DJ, Inga A, Menendez D et al (2005) Functionally distinct polymorphic sequences in the human genome that are targets for p53 transactivation. Proc Natl Acad Sci USA 102:6431–6436
Ueda M, Yamamoto M, Nunobiki O et al (2009) Murine double-minute 2 homolog single nucleotide polymorphism 309 and the risk of gynecologic cancer. Hum Cell 22:49–54
Varley JM (2003) Germline TP53 mutations and Li-Fraumeni syndrome. Hum Mutat 21: 313–320
Varley JM, Evans DG, Birch JM (1997) Li-Fraumeni syndrome—a molecular and clinical review. Br J Cancer 76:1–14
Vazquez A, Bond EE, Levine AJ et al (2008) The genetics of the p53 pathway, apoptosis and cancer therapy. Nat Rev Drug Discov 7:979–987
Vazquez A, Kulkarni D, Grochola LF et al (2011) A genetic variant in a PP2A regulatory subunit encoded by the PPP2R2B gene associates with altered breast cancer risk and recurrence. Int J Cancer 128:2335–2343. doi:10.1002/ijc.25582
Villani A, Tabori U, Schiffman J et al (2011) Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: a prospective observational study. Lancet Oncol 12:559–567. doi:S1470-2045(11)70119-X [pii] 10.1016/S1470-2045(11)70119-X

Vousden KH, Prives C (2009) Blinded by the light: the growing complexity of p53. Cell 137:413–431. doi:S0092-8674(09)00459-0 [pii] 10.1016/j.cell.2009.04.037

Wade M, Wang YV, Wahl GM (2010) The p53 orchestra: Mdm2 and Mdmx set the tone. Trends Cell Biol 20:299–309. doi:S0962-8924(10)00033-4 [pii] 10.1016/j.tcb.2010.01.009

Wallace-Brodeur RR, Lowe SW (1999) Clinical implications of p53 mutations. Cell Mol Life Sci 55:64–75

Wan Y, Wu W, Yin Z et al (2011) MDM2 SNP309, gene-gene interaction, and tumor susceptibility: an updated meta-analysis. BMC Cancer 11:208

Wei MC, Zong WX, Cheng EH et al (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292:727–730. doi:10.1126/science.1059108 292/5517/727 [pii]

Whibley C, Pharoah PD, Hollstein M (2009) p53 polymorphisms: cancer implications. Nat Rev Cancer 9:95–107

Zhang L, Yu J, Park BH et al (2000) Role of BAX in the apoptotic response to anticancer agents. Science 290:989–992. doi:8940 [pii]

Zhou BB, Elledge SJ (2000) The DNA damage response: putting checkpoints in perspective. Nature 408:433–439. doi:10.1038/35044005

Zhou BP, Liao Y, Xia W et al (2001) HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. Nat Cell Biol 3:973–982. doi:10.1038/ncb1101-973 ncb1101-973 [pii]

Zhou M, Gu L, Findley HW et al (2003) PTEN reverses MDM2-mediated chemotherapy resistance by interacting with p53 in acute lymphoblastic leukemia cells. Cancer Res 63:6357–6362

Zhu F, Dolle ME, Berton TR et al (2010) Mouse models for the p53 R72P polymorphism mimic human phenotypes. Cancer Res 70:5851–5859