Breast Cancer Research and Treatment **32**: 39-47, 1994.

The p53 tumor suppressor gene in breast cancer

Richard M. Elledge and D. Craig Allred
Department of Medicine/Medical Oncology and Department of Pathology, University of Texas Health Science Center, San Antonio, Texas, USA

Key words: breast cancer, p53 gene, p53 protein, prognosis, therapy

Summary

Alterations of the p53 tumor suppressor gene are the most common genetic changes found so far in breast cancer, suggesting that the gene plays a central role in the development of the disease. p53 functions as a negative regulator of cell growth, and alterations in the gene lead to loss of this negative growth regulation and more rapid cell proliferation. A number of independent groups using different methods of detection have shown that p53 alterations are associated with more aggressive tumor biologic factors and a poorer prognosis in breast cancer patients. Because of its possible role in the regulation of apoptosis and response to DNA damage, p53 status could also be a predictive marker for response to hormonal or chemotherapy.

Introduction

The pathway to epithelial neoplasia is marked by a series of genomic changes that include mutation, amplification, loss, and rearrangement of DNA. A growing number of oncogenes and tumor suppressor genes are known to be involved in this pathway, but until recently, no one particular gene could be identified that played a common or central role in this seemingly chaotic and random process. The p53 gene has now emerged as the leading candidate for this distinction. p53 mutation is the most common genetic change found in a wide variety of malignancies [1], including breast cancer [2]. This high rate of mutation suggests that the gene plays a central role in neoplastic development in general, and in breast cancer in particular.

p53 functions as a negative regulator of cell growth [3,4] and also inhibits transformation [5]. Evidence points to its role as a sequence-specific [6,7] and non-specific [8] transcription factor which might increase transcription [9] of growth inhibitory genes or repress transcription [8,10] of genes that promote cell growth. More specifically, p53 may play a role in apoptosis [11,12], maintenance of genomic integrity [13,14], and cell differentiation [15]. Cells with p53 mutations show more genomic instability [16] and may be less able to repair DNA damage, differentiate, or undergo apoptosis. It has been hypothesized that p53, acting as a control at a G1 checkpoint [17], may detect DNA damage, slow the cell, and allow time for DNA repair. If damage is irreparable, the cell may be driven down the apoptotic pathway, thus preventing replication of defective cells.

Address for correspondence and offprints: Richard M. Elledge, M.D., Medicine/Oncology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7884, USA; *Tel:* 210-567-4777; *Fax:* 210-567-6687

Table 1. Prognostic studies of p53 in breast cancer

Reference	n	Assay	Rate alt. p53	DFS and/or OS in alt. p53
Iwaya et al [37]	73	IHC*	23%	reduced
Thor et al [23]	295	IHC	23%	reduced
Isola et al [41]	259	IHC	28%	reduced
Allred et al [29]	700	IHC	52%	reduced
Noguchi et al [44]	105	IHC	18%	reduced
Barnes et al [39]	195	IHC	19%	reduced
Silvestrini et al [42]	256	IHC	44%	reduced
Elledge et al [30]	200	SSCP*	14%	reduced
Allred et al [43]	926	IHC	52%	reduced
Thorlacius et al [46]	109	CDGE*	16.5%	reduced
Total	3,118			
Ostrowski et al [40]	90	IHC	36%	ns*
Hanzal et al [38]	117	IHC	25%	ns
Total	207			

* IHC: immunohistochemistry; SSCP: single-strand conformation polymorphism analysis; CDGE: constant denaturing gel electrophoresis; ns: not statistically significant

Mutation or alteration in the gene lead to loss of this negative growth regulation, and thus to more rapid cell proliferation [18]. Also, when mutant p53 is introduced into cells, transformation and a more aggressive phenotype can result [19, 20]. Thus, while wild-type p53 functions as a tumor suppressor gene, mutant p53 can act as a dominant oncogene.

p53 in breast cancer

p53 mutations are common in breast cancer and have been reported at a rate of 15-50%, depending on the stage of disease and the method of detection [21-26]. As with other tumor types, non-invasive or less advanced breast cancer has a lower incidence of alterations [21-23] — for *in situ* disease the incidence of mutation is about 15%, while for invasive and metastatic disease, it is 2 to 3 times higher.

The reported frequency of p53 alterations is dependent on the method of detection. Immunohistochemical staining (IHC) is a protein-based method that is used commonly to detect alterations in p53 because it is relatively inexpensive and easy to perform, especially on a large number of tumors. A mutation results in a prolonged protein half-life and accumulation of the protein in the nucleus [27]. IHC detects this abnormal accumulation and is therefore thought to be an indirect indication of a mutation. 30-50% of breast tumors have accumulation of p53 protein as measured by IHC [23,25,28,29]. DNA-based methods detect fewer alterations, on the order of 15-45% [2,30-32]. The reason for this discrepancy is unclear. DNA-based methods may not be as sensitive; however, one study demonstrated that single-strand conformation polymorphism analysis (SSCP) can detect p53 mutations even when the background wild-type allele comprises as much as 85-95% of the total population of p53 alleles [33]. Alternatively, the mutation could occur outside of the gene regions surveyed.

On the other hand, IHC may overestimate the frequency of gene alterations. Stabilization or accumulation of p53 protein could occur for reasons other than a mutation. Indeed, some have observed nuclear accumulation of protein, but detected no mutation by sequencing [34]. The level of p53 protein normally increases during late G1 and S-phase [35], and also increases in res-

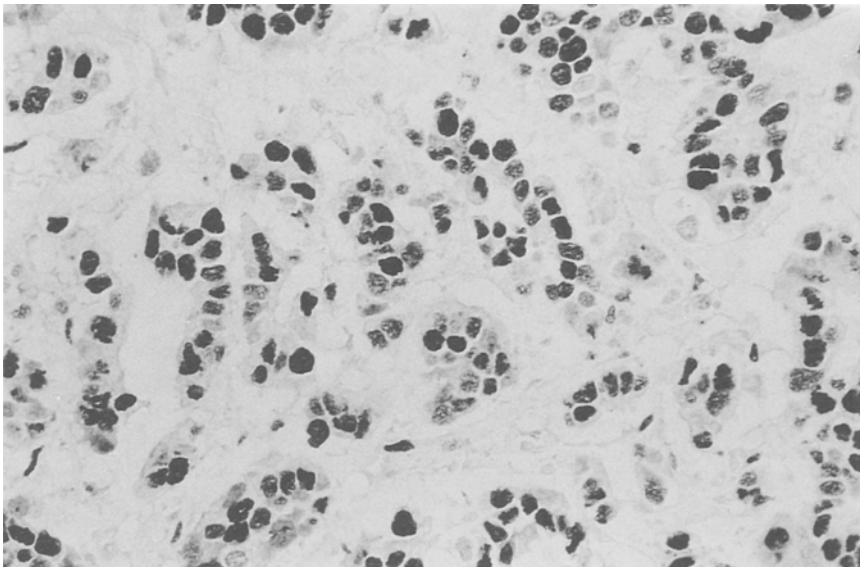

Figure 1. Example of an infiltrating ductal carcinoma which stains positively for nuclear p53 protein. Frozen section IHC was performed with a cocktail of the antibodies 1801 and 240. Surrounding small lymphocytes are negative.

ponse to DNA damage [13]. Cells that are in these phases of the cell cycle or are genetically damaged may have positive staining without a mutation. Accumulation of p53 could also occur as a result of binding to other cellular proteins. p53 is known to bind to mdm-2 protein [36], a cellular oncoprotein, as well as several other viral proteins which could have cellular homologues.

Testing for p53 alterations may have a prognostic clinical application. Alterations in the gene lead to loss of its negative growth regulatory function, and hence to a more rapid cell proliferation. Also, p53 alterations are more often found in more advanced breast cancer. This suggests the possibility that p53 alterations occur more often as a late event in the transformation process, or are associated with an increased metastatic potential. For these reasons and because p53 mutations are common in breast cancer, it has been thought by ourselves and others that p53 mutations could be associated with more aggressive tumors or those with higher likelihood of occult distant metastasis, and thus might be a prognostic factor in predicting future recurrence. Also, because of p53's involvement in the regulation of the cell cycle, apoptosis, and response to DNA damage, p53 status might be used as a predictor of response to cytotoxic or hormonal therapy.

p53 status and breast cancer prognosis

A number of studies involving over 3,000 patients have examined the relationship between the nuclear accumulation of p53 protein, prognosis (Table 1), and known adverse biologic factors [23,29,37-44]. These studies have used different antibodies and methodologies and were performed on both frozen and paraffin-embedded formalin-fixed specimens. This probably accounts for the reported range of positive staining. From these studies, it appears that frozen section staining is more sensitive than permanent section IHC, and that a combination of antibodies directed against different epitopes may increase sensitivity. An example of frozen-section IHC done by our group, utilizing a cocktail of the antibodies 1801 and 240, is shown in Figure 1.

Of 10 published series, 8 show a poorer out-

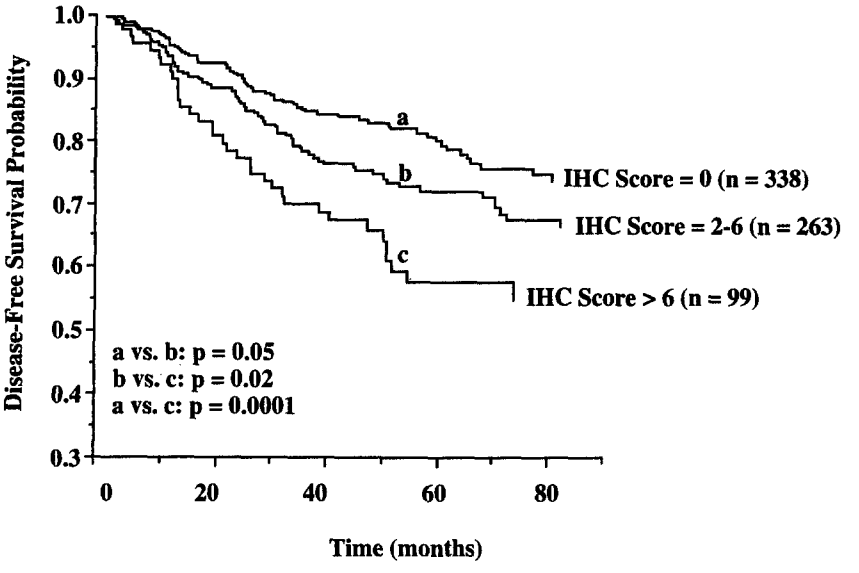

Figure 2. Disease-free survival of 700 node-negative patients according to p53 IHC status. Tumors were divided into three groups according to IHC score. The overall amount of p53 protein present in each tumor was expressed as the sum of an intensity score (0 to 3) and a proportion score (0 to 5). Tumors were placed into one of 3 groups, negative tumors (IHC score = 0), low positive tumors (IHC score = 2 to 6), and high positive tumors (IHC score > 6). DFS decreased as IHC score increased.

come for women with p53 IHC-positive tumors. In one of the two negative studies, patients with IHC-positive did have a worse survival, but the difference was not statistically significant [40]. This poorer survival is seen in both lymph node-positive and lymph node-negative groups. This is especially important in the latter group, where prognostic factors could help guide therapeutic decisions. We have performed the largest study examining prognosis in lymph node-negative patients. In 700 patients, disease-free survival at 5 years was 80% for negative tumors, compared with 72% for low-positive to intermediate-positive tumors, and 58% for high-positive tumors [29] (Figure 2). Thus, not only does the accumulation of p53 indicate a worse prognosis, but it also appears that as the proportion of p53 abnormal cells in a tumor increases, prognosis becomes worse. Supporting the notion that tumor aggressiveness is directly related to the proportion of IHC-positive cells are molecular experiments which demonstrate that the proportion of p53 mutated cells in a tumor increases as a tumor progresses and is higher after recurrence [45].

Accumulation of p53 protein is associated with a number of other poor prognostic factors, including ER negativity [23,26,29,37,40-42], PgR negativity [29,39,41], high histologic grade [37, 39-41] or nuclear grade [23], erbB-2 over-expression [37,41], aneuploidy [29], and a high proliferative fraction [29,41-43]. No consistent relationship has been seen between p53 status and tumor size. Since p53 functions to control entry or progression through the cell cycle, one would expect that tumors with an inactivated p53 would have a higher rate of proliferation, and this is indeed the case. We have also found that there is a strong direct correlation between the amount of mutant protein present and tumor proliferation rate. Tumors with the highest amount of nuclear p53 had the highest median S-phase values [29]. These results are consistent with the hypothesis that wild-type p53 is involved in suppression of the cell cycle. It has been suggested that p53 IHC status is simply a surrogate marker for S-phase fraction; however, our group and others

have found that p53 status predicts recurrence independent of proliferative measures [29,42]. This corroborates data from molecular studies indicating that p53 is a pleiotropic protein and has functions other than simply controlling cell proliferation.

At least two studies have examined the relationship between p53 gene alteration and prognosis. Each searched for p53 gene alterations in evolutionarily conserved and functionally important regions involving part or all of exons 5 through 9. Most mutations have been found in these regions. To search for gene alterations we used SSCP analysis, an electrophoretic method that can identify single base pair differences in polymerase chain reaction amplified DNA fragments [30]. An example of an SSCP analysis is shown in Figure 3. Double-stranded fragments are seen at the bottom, and normal single-stranded fragments are seen in the upper part of the gel. After denaturation, the tumor in lane 6 shows an additional band representing an altered 8/9 sequence. Later sequence analysis confirmed a point mutation in codon 274. Of 200 node-negative tumors, 1 to 3 cm in size, 14% contained a p53 gene alteration, and disease-free survival was significantly decreased in this group (Figure 4). Survival was 78% at 5 years for SSCP-normal

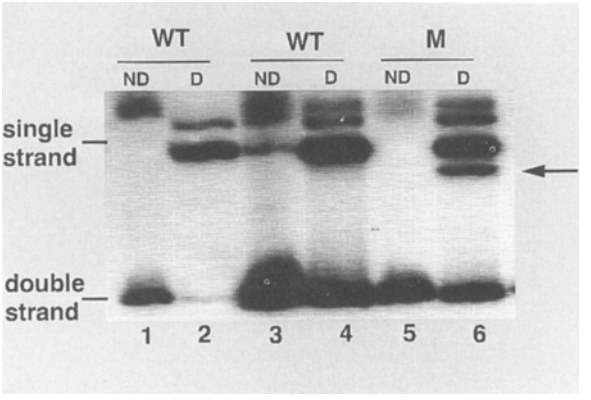

Figure 3. SSCP analysis of p53, exon 8/9. Single-strand conformers are seen at the top and double-strand conformers are seen at the bottom. Samples were run under denaturing (D) and nondenaturing (N) conditions. An abnormally migrating band, representing a GTT to GCT mutation at codon 274, is seen in lane 6. WT: wild type; M: mutation.

tumors, but only 58% for women with SSCP-abnormal tumors. Thorlacius studied 109 tumors with constant denaturant gel electrophoresis and found that 16.5% of the tumors had a mutation [46]. They also found a strong association between a mutation and poor prognosis at 32 months. Similar to IHC studies, both of these DNA-based analyses found an association between ER negativity and p53 gene alterations.

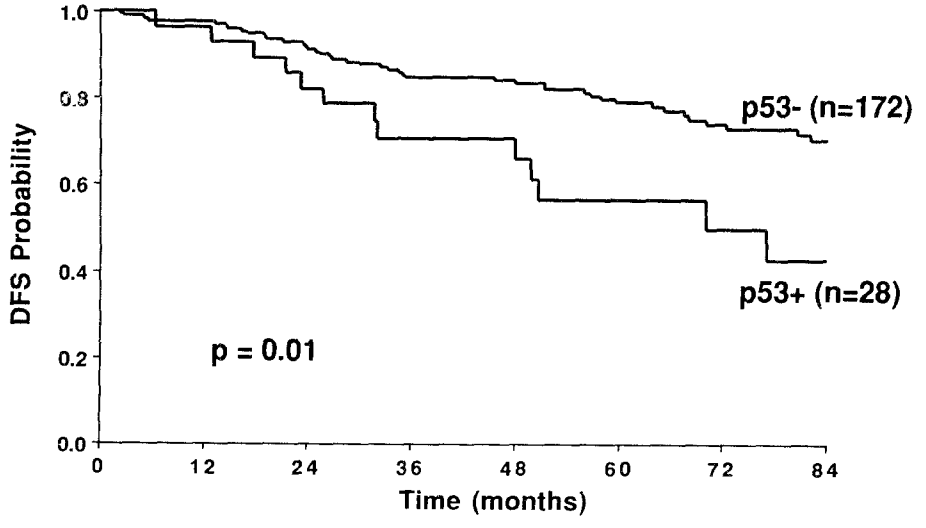

Figure 4. Disease-free survival in patients with node-negative breast cancer, as a function of a normal (p53–) or abnormal (p53+) SSCP analysis for p53, exons 5 through 9.

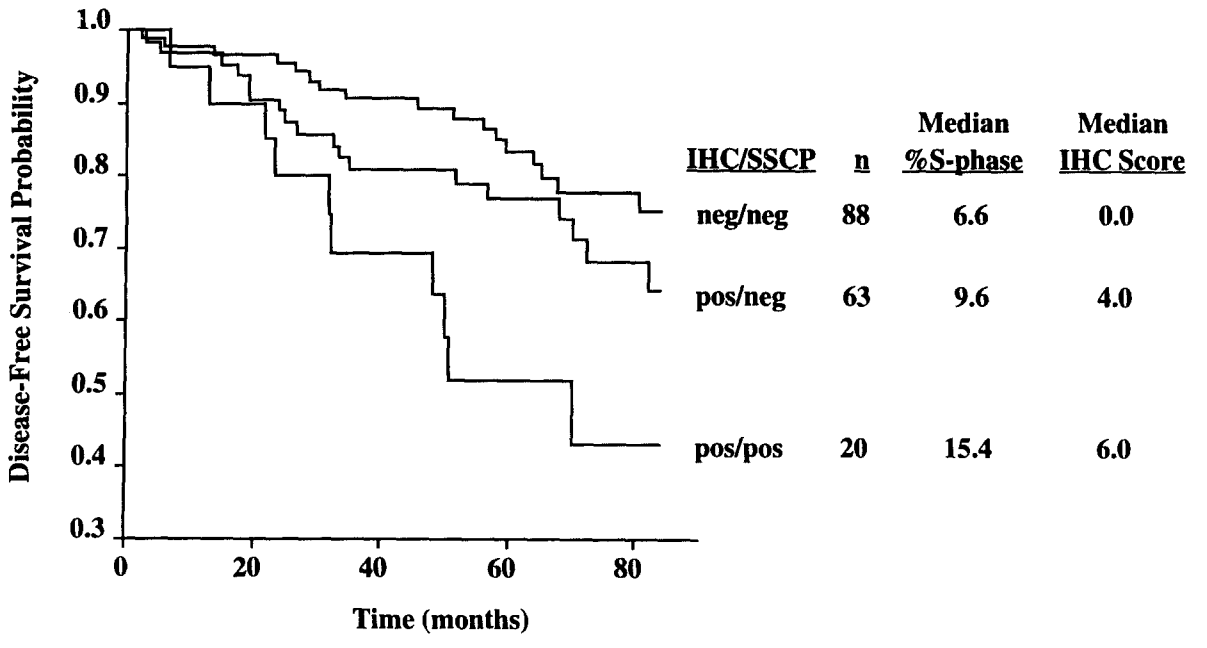

Figure 5. Disease-free survival as a function of altered p53 determined by both IHC and SSCP. SSCP analysis was performed on p53 exons 5–9 in 176 node-negative tumors also studied for p53 protein expression by IHC, and for tumor proliferation by flow cytometry (% S-phase fraction). Five year DFS progressively decreased from tumors that were negative by both IHC and SSCP (85%), to the tumors that were IHC positive and SSCP negative (78%), to the tumors that were positive by both methods (54%). Tumor proliferation rate and over-expression of p53 by IHC also increased in the same order. (Only 5 tumors appeared negative by IHC but positive by SSCP.)

Our study also found that SSCP-abnormal tumors had a higher S-phase fraction compared with tumors having a normal SSCP.

To address the discrepancy between IHC and DNA-based methods, we compared the IHC and SSCP status of 176 tumors directly (Figure 5). 88 tumors were negative by both methods, and these had the best prognosis and lowest S-phase fraction. 20 tumors were positive by both methods and had the worst prognosis and highest S-phase fraction. Those that were positive by IHC and negative by SSCP had an intermediate prognosis and an intermediate S-phase fraction. 5 tumors were SSCP-positive and IHC-negative, and may represent mutations that produce truncations or do not result in amino acid changes. Thus, discordance was common, and IHC detected protein accumulation more commonly than SSCP detected gene alterations. Tumors with increased p53 protein levels but no detectable gene alteration might be explained by epigenetic events such as binding of stabilizing proteins or post-transcriptional modification.

p53 in predicting response to therapy

p53 might also be useful for predicting response to hormonal or chemotherapy in breast cancer. By a number of speculative mechanisms, p53 could alter a breast tumor's responsiveness to systemic therapy. Hormonal or chemotherapy may act by inducing cell apoptosis [47,48], and a cell with loss of p53 function may not be able to undergo apoptosis and thus would be resistant to the effects of hormonal or cytotoxic therapy. Conversely, if p53 acts to detect DNA damage and slow the cell to allow time for repair, cells with a defective p53 may not be able to repair damage as efficiently and therefore would be

more sensitive to chemotherapeutic agents that act by damaging DNA. Hormonal agents act through estrogen receptor to influence cell proliferation and transit through the cell cycle. In the presence of a mutated p53, the influence of estrogen receptor on cell cycle control may be lost and the cell may then be no longer responsive to hormonal agents. Lastly, p53 mutations can alter growth factor interactions thought to be important in the therapeutic response to tamoxifen. Evidence suggests that tamoxifen therapy can produce an inhibitory effect on breast cancer by increasing TGFβ [49], a growth factor that slows breast cancer cell growth. In human bronchial epithelium, mutant p53 causes a loss in the inhibitory response usually seen with TGFβ [50]. Breast cancers with p53 mutation may thus no longer be responsive to the normal growth-retarding effects of TGFβ.

Current and future studies

We have several current and future research plans regarding p53. First, since p53 is a weak prognostic factor, which cannot alone identify a group with such a low risk of recurrence that treatment would not be indicated, we will be integrating p53 in a multifactor prognostic model to determine its relative contribution to prognosis and its interaction with other variables. When multiple factors are combined, the prognosis of subsets of patients may be more accurately defined. Second, the predictive value of p53 will be tested *in vivo*. For this purpose, we will use archival paraffin blocks from SWOG 8228, a prospective study in which 340 patients with ER-positive metastatic breast cancer were given tamoxifen, and the resulting time to progression and survival were recorded. Blocks will be evaluated for p53 and other biologic factors which may be associated with response to tamoxifen. *In vitro* models utilizing MCF-7 cells transfected with mutated p53 will be used in experiments to test for tamoxifen sensitivity and estrogen independent growth. Tumorigenicity and estrogen/tamoxifen responsiveness of p53 transfectants will be evaluated in nude mice. Lastly, other factors could modify p53 activity and its prognostic significance. Heat shock protein 70 is a molecular chaperone which is known to bind mutant [51,52] and possibly wild-type [53] p53. This interaction could modulate the biological effects of p53 through stabilization of the protein or alteration of its subcellular localization. We will therefore be investigating whether hsp 70 co-localizes with p53 protein in human breast tumor cells, and whether it plays a role in p53 accumulation.

Conclusion

In summary, p53 mutations play a central role in breast cancer progression. In studies involving over 3100 patients, multiple independent groups using different methodology have found p53 mutation to be a weak independent prognostic factor for early recurrence in breast cancer. Future studies will indicate whether it is also a predictive factor for response to endocrine or chemotherapy.

Acknowledgement

Supported by NIH SPORE P50 CA 58183 and program project CA 30195. RM Elledge is the recipient of a SPORE Career Development Award. We wish to thank Gladys Locsos for assistance in preparing this manuscript.

References

1. Hollstein M, Sidransky D, Vogelstein B, Harris CC: p53 mutations in human cancers. Science 253:252-254, 1991
2. Osborne RJ, Merlo GR, Mitsudomi T, Venesio T, Liscia DS, Cappa APM, Chiba I, Takahashi T, Nau MM, Callahan R, Minna JD: Mutations in the p53 gene in primary human breast cancers. Cancer Res 51:6194-6198, 1991
3. Noble JR, Willetts KE, Mercer WE, Reddel RR: Effects of exogenous wild-type p53 on a human lung carcinoma cell line with endogenous wild-type p53. Exper Cell

Res 203:297-304, 1992
4. Cajot J-F, Anderson MJ, Lehman TA, Shapiro H, Briggs AA, Stanbridge EJ: Growth suppression mediated by transfection of p53 in Hut292DM human lung cancer cells expressing endogenous wild-type p53 protein. Cancer Res 52:6956-6960, 1992
5. Finlay CA, Hinds PW, Levine AJ: The p53 proto-oncogene can act as a suppressor of transformation. Cell 57:1083-1093, 1989
6. Funk WD, Pak DT, Karas RH, Wright WE, Shay JW: A transcriptionally active DNA-binding site for human p53 protein complexes. Molec Cell Biol 12:2866-2871, 1992
7. Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P, Prives C, Vogelstein B: Identification of p53 as a sequence-specific DNA-binding protein. Science 252: 1708-1711, 1991
8. Mack DH, Vartikar J, Pipas JM, Laimins LA: Specific repression of TATA-mediated but not initiator-mediated transcription by wild-type p53. Nature 363:281-283, 1993
9. Fields S, Jang SK: Presence of a potent transcription activating sequence in the p53 protein. Science 249: 1046-1049, 1990
10. Seto E, Usheva A, Zambetti GP, Momand J, Horikoshi N, Weinmann R, Levine AJ, Shenk T: Wild-type p53 binds to the TATA-binding protein and represses transcription. Proc Natl Acad Sci USA 89:12028-12032, 1992
11. Ramqvist T, Magnusson KP, Wang Y, Szekely L, Klein G, Wiman KG: Wild-type p53 induces apoptosis in a Burkitt lymphoma (BL) line that carries mutant p53. Oncogene 8:1495-1500, 1993
12. Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M: Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 352:345-347, 1991
13. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW: Participation of p53 protein in the cellular response to DNA damage. Cancer Res 51:6304-6311, 1991
14. Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD: Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70:923-935, 1992
15. Feinstein E, Gale RP, Reed J, Canaani E: Expression of the normal p53 gene induces differentiation of K562 cells. Oncogene 7:1853-1857, 1992
16. Yin Y, Tainsky MA, Bischoff FZ, Strong LC, Wahl GM: Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell 70:937-948, 1992
17. Lin D, Shields MT, Ullrich SJ, Appella E, Mercer WE: Growth arrest induced by wild-type p53 protein blocks cells prior to or near the restriction point in late G_1 phase. Proc Natl Acad Sci USA 89:9210-9214, 1992
18. Michalovitz D, Halevy O, Oren M: Conditional inhibition of transformation and of cell proliferation by a temperature-sensitive mutant of p53. Cell 62:671-680, 1990
19. Rovinski B, Benchimol S: Immortalization of rat embryo fibroblasts by the cellular p53 oncogene. Oncogene 2:445-452, 1988
20. Jenkins JR, Rudge K, Currie GA: Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. Nature 312:651-654, 1984
21. Tsuda H, Iwaya K, Fukutomi T, Hirohashi S: p53 mutations and c-erbB-2 amplification in intraductal and invasive breast carcinomas of high histologic grade. Jpn J Cancer Res 84:394-401, 1993
22. Davidoff AM, Kerns B-JM, Pence JC, Marks JR, Iglehart JD: p53 alterations in all stages of breast cancer. J Surg Oncol 48:260-267, 1991
23. Thor AD, Moore DH II, Edgerton SM, Kawasaki ES, Reihsaus E, Lynch HT, Marcus JN, Schwartz L, Chen L-C, Mayall BH, Smith HS: Accumulation of p53 tumor suppressor gene protein: An independent marker of prognosis in breast cancer. J Natl Cancer Inst 84:845-855, 1992
24. Runnebaum IB, Nagarajan M, Bowman M, Soto D, Sukumar S: Mutations in p53 as potential molecular markers for human breast cancer. Proc Natl Acad Sci USA 88:10657-10661, 1991
25. Bártek J, Bártková J, Voitesek B, Stasková Z, Rejthar A, Kovarik J, Lane DP: Patterns of expression of the p53 tumour suppressor in human breast tissues and tumours *in situ* and *in vitro*. Int J Cancer 46:839-844, 1990
26. Mazars R, Spinardi L, BenChikh M, Simony-Lafontaine J, Jeanteur P, Theillet C: p53 mutations occur in aggressive breast cancer. Cancer Res 52:3918-3923, 1992
27. Davidoff AM, Humphrey PA, Iglehart JD, Marks JR: Genetic basis for p53 overexpression in human breast cancer. Proc Natl Acad Sci USA 88:5006-5010, 1991
28. Davidoff AM, Kerns B-JM, Iglehart JD, Marks JR: Maintenance of p53 alterations throughout breast cancer progression. Cancer Res 51:2605-2610, 1991
29. Allred DC, Clark GM, Elledge R, Fuqua SAW, Brown RW, Chamness GC, Osborne CK, McGuire WL: Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. J Natl Cancer Inst 85:200-206, 1993
30. Elledge RM, Fuqua SAW, Clark GM, Pujol P, Allred DC, McGuire WL: Prognostic significance of p53 gene alterations in node-negative breast cancer. Breast Cancer Res Treat 26:225-235, 1993

31. Marchetti A, Buttitta F, Pellegrini S, Campani D, Diella F, Cecchetti D, Callahan R, Bistocchi M: p53 mutations and histological type of invasive breast carcinoma. Cancer Res 53:4665-4669, 1993
32. Coles C, Condie A, Chetty U, Steel CM, Evans J, Prosser J: p53 mutations in breast cancer. Cancer Res 52:5291-5298, 1992
33. Wu JK, Ye Z, Darras BT: Sensitivity of single-strand conformation polymorphism (SSCP) analysis in detecting p53 point mutations in tumors with mixed cell populations. Am J Hum Genet 52:1273-1275, 1993
34. Barnes DM, Hanby AM, Gillett CE, Mohammed S, Hodgson S, Bobrow LG, Leigh IM, Purkis T, MacGeoch C, Spurr NK, Bartek J, Vojtesek B, Picksley SM, Lane DP: Abnormal expression of wild type p53 protein in normal cells of a cancer family patient. Lancet 340:259-263, 1992
35. Shaulsky G, Ben-Ze'ev A, Rotter V: Subcellular distribution of the p53 protein during the cell cycle of Balb/c 3T3 cells. Oncogene 5:1701-1711, 1990
36. Momand J, Zambetti GP, Olson DC, George D, Levine AJ: The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 69:1237-1245, 1992
37. Iwaya K, Tsuda H, Hiraide H, Tamaki K, Tamakuma S, Fukutomi T, Mukai K, Hirohashi S: Nuclear p53 immunoreaction associated with poor prognosis of breast cancer. Jpn J Cancer Res 82:835-840, 1991
38. Hanzal E, Gitsch G, Kohlberger P, Dadak CH, Miechowiecka N, Breitenecker G: Immunohistochemical detection of mutant p53-suppressor gene product in patients with breast cancer: Influence on metastasis-free survival. Anticancer Res 12:2325-2330, 1992
39. Barnes DM, Dublin EA, Fisher CJ, Levison DA, Millis RR: Immunohistochemical detection of p53 protein in mammary carcinoma: An important new independent indicator of prognosis? Hum Pathol 24:469-476, 1993
40. Ostrowski JL, Sawan A, Henry L, Wright C, Henry JA, Hennessy C, Lennard JW, Angus B, Horne HW: p53 expression in human breast cancer related to survival and prognostic factors: An immunohistochemical study. J Pathol 164:75-81, 1991
41. Isola J, Visakorpi T, Holli K, Kallioniemi O-P: Association of overexpression of tumor suppressor protein p53 with rapid cell proliferation and poor prognosis in node-negative breast cancer patients. J Natl Cancer Inst 84:1109-1114, 1992
42. Silvestrini R, Benini E, Daidone MG, Veneroni S, Boracchi P, Cappelletti V, Di Fronzo G, Veronesi U: p53 as an independent prognostic marker in lymph node-negative breast cancer patients. J Natl Cancer Inst 85:965-970, 1993
43. Allred DC, Clark GM, Fuqua SAW, Elledge RM, Hilsenbeck SG, Ravdin PM, Yee D, Chamness GC, Osborne CK: Overexpression of p53 in node-positive breast cancer. Breast Cancer Res Treat 27:131, 1993
44. Noguchi M, Kitagawa H, Kinoshita K, Miyazaki I, Saito Y, Mizukami Y: Prognostic significance of p53 and c-erbB-2 experience in operable breast cancer. Int J Oncol (Greece) 2:587-591, 1993
45. Sidransky D, Mikkelsen T, Schwechheimer K, Rosenblum ML, Cavanee W, Vogelstein B: Clonal expansion of p53 mutant cells is associated with brain tumour progression. Nature 355:846-847, 1992
46. Thorlacius S, Börresen A-L, Eyfjörd E: Somatic p53 mutations in human breast carcinomas in an Icelandic population: A prognostic factor. Cancer Res 53:1637-1641, 1993
47. Wilson AC, Singh M, Thompson HJ: Morphological responses of MOD cells to tamoxifen suggests induction of apoptosis. Proc Am Asso Cancer Res 33:151, 1993
48. Kyprianou N, English HF, Davidson NE, Isaacs JT: Programmed cell death during regression of the MCF-7 human breast cancer following estrogen ablation. Cancer Res 51:162-166, 1991
49. Knabbe C, Lippman ME, Wakefield LM, Flanders KC, Kasid A, Derynck R, Dickson RB: Evidence that transforming growth factor-β is a hormonally regulated negative growth factor in human breast cancer cells. Cell 48:417-428, 1987
50. Gerwin BI, Spillare E, Forrester K, Lehman TA, Kispert S, Welsh JA, Pfeifer AMS, Lechner JF, Baker LJ, Vogelstein B, Harris CC: Mutant p53 can induce tumorigenic conversion of human bronchial epithelial cells and reduce their responsiveness to a negative growth factor, transforming growth factor β1. Proc Natl Acad Sci USA 89:2759-2763, 1992
51. Finlay CA, Hinds PW, Tan T-H, Eliyahu D, Oren M, Levine AJ: Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. Mol Cell Biol 8:531-539, 1988
52. Yehiely F, Oren M: The gene for the rat heat-shock cognate, *hsc70*, can suppress oncogene-mediated transformation. Cell Growth Diff 3:803-809, 1992
53. Lehman TA, Bennett WP, Metcalf RA, et al: p53 mutations, *ras* mutations, and p53-heat shock protein 70 complexes in human lung carcinoma cell lines. Cancer Res 51:4090-4096, 1991