Annals of Diagnostic Pathology 21 (2016) 29–34

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

p53 expression and relationship with MDM2 amplification in breast carcinomas

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ARTICLE INFO

Keywords:
p53
MDM2
Breast carcinoma

ABSTRACT

Carcinoma of the breast, like other malignancies, is a genetic disease with multiple genetic events leading to the malignant phenotype. p53 mutations are the most common genetic events in human cancer. Inactivation of p53 can be a result of mutation in gene sequence. One of the main structures that regulate p53 stabilization is MDM2. It suppresses p53 transcriptional activation by recognizing transactivation domain of p53. The loss of MDM2 function on p53 regulation results in deprivation of p53 tumor suppressor ability. Single nucleotide polymorphisms (SNP309 T->G exchange) or MDM2 amplification has been proposed to play a role in this issue. In the present study, our aim is to analyze p53 and MDM2 status and investigate their interactions in human sporadic breast carcinoma. The study groups were separated according to their molecular classifications. In each group, histologic type of the tumor, conventional prognostic parameters, p53, and MDM2 interactions were compared statistically. Tumors are divided into 4 subtypes due to estrogen and progesterone receptor status, HER-2, and Ki-67 proliferation index results. According to this classification, 23 cases are in the luminal A, 32 cases are in the luminal B, 15 cases are in the HER-2 positive, and 22 cases are in the triple-negative group, with a total of 92 cases. p53 expression is low in luminal breast carcinomas than HER-2 and triple-negative subtypes. MDM2 amplification frequency was found to be 5.4% in total. MDM2 gene amplification does not have a significant role in breast carcinogenesis, but other possible mechanisms may play a role in its inactivation.

1. Introduction

Breast carcinoma is the most common malignant tumor and the leading cause of death from carcinoma in women, with more than 1 000 000 cases occurring worldwide annually. In Western developed countries such as the United States, the lifetime risk for breast cancer is 1 in 8 women approximately.

The best known risk factor of breast cancer is strong and prolonged estrogen stimulation. Family history; menstrual and reproductive history; fibrocystic changes and epithelial hyperplasia; exogenous estrogens; contraceptive agents; ionizing radiation; country of birth (Far East); and some syndromes such as Li-Fraumeni, Cowden, and ataxia telengiectasia are other well-known risk factors [1,2].

BRCA1 and BRCA2 genes are responsible for two-third of familial breast carcinomas. BRCA1 mutation is a predisposing factor for breast, ovary, and fallopian tube carcinomas and associated with bad prognosis [1,2]. Tumor size is directly correlated with prognosis. Microscopic grade, status of margins, and lymph node metastasis also dictate the prognosis [1,2].

Gene expression studies make a great progress in recent years that evaluation of expression of thousands of genes through microarray technology is currently possible; thereby, the separation of the prognostic groups has become more distinct. Breast carcinomas can be divided according to the gene expression profile into, luminal type, HER-2–positive type, basal-like type, and normal breast tissue such as type breast carcinomas [1,2].

In comparison with molecular markers such as hormone receptors, HER-2 status, p53, Ki-67, bcl-2 expression, and DNA ploidy, estrogen receptor (ER)–positive breast carcinomas have a longer survival. Overexpression of HER-2 oncogene is an excellent predictor of response to Herceptin but a weaker predictor for chemotherapy. p53 accumulation, which is presumably related with the p53 gene mutation, is associated with reduced survival. It has also been shown that loss of heterozygosity for p53 is strongly associated with high histologic and nuclear grade [1,2].

Gene p53, located at chromosome 17, is a tumor suppressor gene that encodes p53 protein. It is frequently mutated in sporadic cancers. p53 function can be compromised by various mechanisms as mutations

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http://dx.doi.org/10.1016/j.anndiagpath.2016.01.001
1092-9134/

in target genes of p53 or TP53 itself and changes in p53 regulatory genes and proteins [3-8]. More than 20 000 p53 variations were estimated in human tumors, whereas 30% of breast tumors have p53 mutation [9].

Breast tumors expressing a high amount of p53, as measured by immunohistochemistry, are more frequently ER negative and progesterone receptor (PR) negative. They are also associated with a high proliferation rate, high histologic and nuclear grades, aneuploidy, and poor survival. Mutations in the p53 gene occur more frequently in HER-2–amplified tumors [3,5,10,11].

p53 mutations may help to identify a subset of very high-risk breast cancer patients with worse prognosis. Data also suggest that the nature of the p53 mutation influences sensitivity to cytotoxic drugs [12].

p53 mutation is quite low in breast cancer compared with other human carcinomas. Approximately 80% of cases have wild-type p53. p53 mutation is estimated in 60% to 65% of lung and colon cancers; 40% to 45% of esophagus, stomach, and bladder cancers; 25% to 30% of breast, liver, and prostate cancers and lymphoma; 24.5% of renal cell cancer; and 10% to 15% of leukemia [12].

Estrogen receptor–positive breast cancers, approximately 75% to 80% of all breast cancers, often have wild-type p53 which have dysfunctional p53. Estrogen receptor–negative breast cancers usually have mutant p53. Therefore, there are different mechanisms that change p53 signal pathway in ER-positive and ER-negative breast cancer [13].

Breast cancer cells with wild-type *p53* often have high levels of the oncogenic protein MDM2 suggesting that MDM2 might block the function of p53. MDM2 is a significant regulator for p53 tumor suppression [13].

The MDM2 oncogene is amplified in a substantial proportion of ER+ early stage breast carcinomas and an independent parameter for poor patient outcome in this subgroup. The prognostic effect of MDM2 is closely connected to ER expression of breast carcinomas [14].

Estrogen-induced breast cancer cell proliferation required a p53-independent role of MDM2 which is a strong contributor to the bypass of cell cycle checkpoints [15].

Treatments of breast cancer continue to evolve rapidly. Restoring p53 activity by inhibiting the interaction between p53 and MDM2 represents an attractive approach for cancer therapy. At this point, a small molecule that inhibits p53-MDM2 binding has been developed during the past several years. Nutlin-3 is a potent and selective MDM2 antagonist [13]. Especially, in the early stages of ER-positive breast cancer, MDM2 amplification may be searched for an alternative treatment.

2. Materials and methods

In this research, 92 samples were obtained from patients diagnosed with breast cancer from 2012 to 2014. Clinicopathologic data were collected from the medical records of patients. Ethical committee approval was obtained (B.30.2.BAV.0.05.05/474).

Formalin-fixed, paraffin-embedded breast tissue samples obtained from patients were examined with hematoxylin-eosin staining as well as by immunohistochemical (IHC) evaluation with estrogen (monoclonal rabbit antibody; Thermo, Fremont, CA) and progesterone (monoclonal rabbit antibody; Thermo) receptors, antibodies against HER-2 protein (c-erb B2) (monoclonal mouse antibody; Thermo), p53 (monoclonal mouse antibody; Biogenex, Fremont, CA) expression, and Ki-67 (monoclonal mouse antibody; Biocare, Concord, CA) proliferation index. Immunohistochemical studies were performed automatically with Ventana Bencmark XT (Tucson, AZ). MDM2 gene amplification was studied with chromogenic in situ hybridization (CISH) techniques as manually with MDM2 probe (ZytoDot SPEC MDM2 Probe; Zytovision, Bremerhaven, Germany). Results were evaluated according to the conventional histologic types and classical prognostic parameters as well as molecular subtypes due to immunohistochemistry: luminal A and B, HER-2–positive, and triple-negative breast cancers.

Table 1
There is a significant correlation between tumor groups and p53 expression, $P = .024$ (<.05).

			p53		Total
			(−)	(+)	
Groups	Luminal A	Count	19	4	23
		% within groups	82.6%	17.4%	100.0%
	Luminal B	Count	22	10	32
		% within groups	68.8%	31.3%	100.0%
	HER-2 (+)	Count	8	7	15
		% within groups	53.3%	46.7%	100.0%
	Triple (−)	Count	9	13	22
		% within groups	40.9%	59.1%	100.0%
Total		Count	58	34	92
		% within groups	63.0%	37.0%	100.0%
χ^2 Tests					
	Value	*P*			
Pearson χ^2	9.459[a]	.024			

[a] Zero cells (0.0%) have expected count less than 5. The minimum expected count is 5.54.

Tumors are divided into 4 subtypes due to estrogen and progesterone receptors, HER-2, and Ki-67 proliferation index results. Estrogen receptor– and/or PR-positive, HER-2–negative, and Ki-67 index less than 20% ones are in luminal A; ER- and/or PR-positive, HER-2–negative or HER-2–positive, and Ki-67 index higher than 20% ones are in luminal B; ER/PR-negative and HER-2–positive (immunohistochemically +3 or immunohistochemically +2 and positive with in situ hybridization) ones are in HER-2–rich group; and ER/PR/HER-2–negative ones are in triple-negative group. According to this classification, 23 cases were in the luminal A, 32 cases were in the luminal B, 15 cases were in the HER-2 positive, and 22 cases were in the triple-negative group. Anti-p53 antibody application in immunohistochemistry and CISH techniques were applied to tumor samples in all cases, to evaluate p53 status and MDM2 amplification. Every group was analyzed in terms of patient age, tumor size, tumor type, histologic grade, multifocality, lymphovascular invasion, lymph node metastasis, p53 expression, and MDM2 amplification. Pearson χ^2 test and Fisher exact test were used for statistical assessment.

3. Results

All groups were compared in terms of p53 expression. Although p53 expression rates are 17.4% and 31.2% in luminal A and B, p53 expression

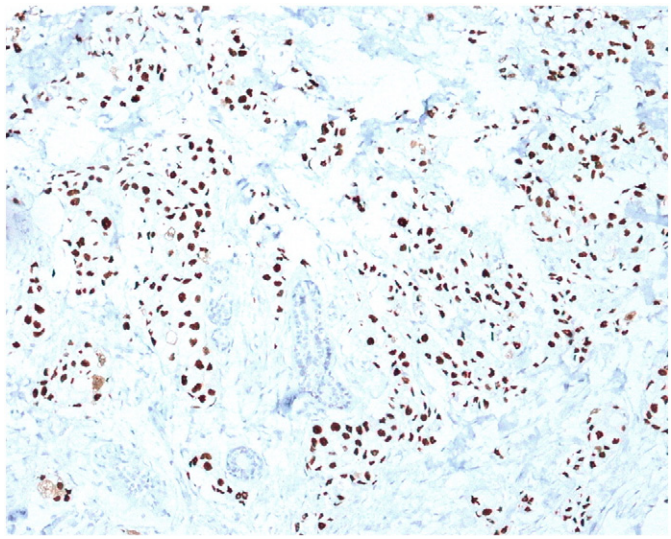

Fig. 1. Intense p53 expression in HER-2 tumor group (×100).

Table 2
MDM2 amplification is established in 5 cases among all breast carcinomas.

			MDM2 amplification (CISH)		Total
			(−)	(+)	
Groups	Luminal A	Count	22	1	23
		% within groups	95.7%	4.3%	100.0%
	Luminal B	Count	29	3	32
		% within groups	90.6%	9.4%	100.0%
	HER-2 (+)	Count	15	0	15
		% within groups	100.0%	0.0%	100.0%
	Triple (−)	Count	21	1	22
		% within groups	95.5%	4.5%	100.0%
Total		Count	87	5	92
		% within groups	94.6%	5.4%	100.0%

MDM2 rates of each group are shown in the table. Statistical analysis was not possible due to the limited number of the MDM2-positive cases.

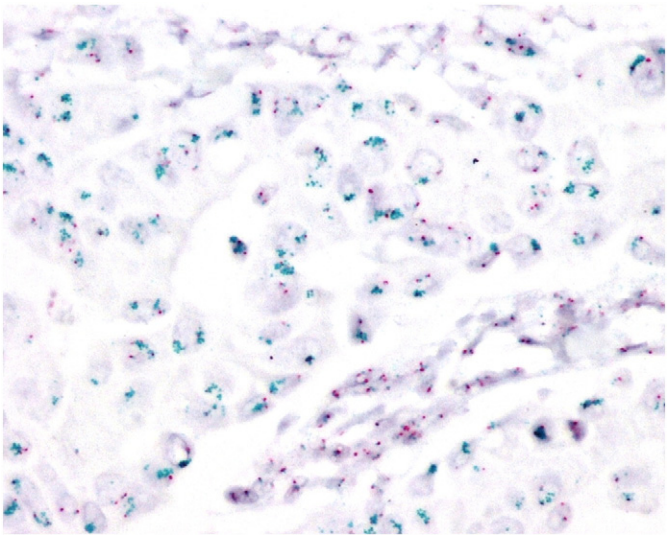

Fig. 2. MDM2 gene amplification in luminal B tumor group (×600).

rates were 53.3% and 40.9% in HER-2 and triple-negative group, respectively ($P = .024$ [<.05]) (Table 1; Fig. 1).

MDM2 amplification rates of groups were in order of 1 (4.3%), 3 (9.4%), 0 (0.0%), 1 (4.5%), and 5 cases (5.4%) in total. Although positive case rate was not statistically significant, it can be concluded that MDM2 amplification was not closely connected to the breast cancer development (Table 2; Fig. 2).

Table 3
There is not a significant correlation between tumor groups and patient age (cutoff, 50), $P = .233$.

			Age		Total
			<50	>50	
Groups	Luminal A	Count	6	17	23
		% within groups	26.1%	73.9%	100.0%
	Luminal B	Count	16	16	32
		% within groups	50.0%	50.0%	100.0%
	HER-2 (+)	Count	5	10	15
		% within groups	33.3%	66.7%	100.0%
	Triple (−)	Count	11	11	22
		% within groups	50.0%	50.0%	100.0%
Total		Count	38	54	92
		% within groups	41.3%	58.7%	100.0%
	Value	*P*			
Pearson χ^2	4.274[a]	.233			

[a] Zero cells (0.0%) have expected count of less than 5. The minimum expected count is 6.20.

Table 4
There is not a significant correlation between tumor groups and histologic types.

			Tumor type		Total
			Other	IDC	
Groups	Luminal A	Count	9	14	23
		% within groups	39.1%	60.9%	100.0%
	Luminal B	Count	5	27	32
		% within groups	15.6%	84.4%	100.0%
	HER-2 (+)	Count	3	12	15
		% within Groups	20.0%	80.0%	100.0%
	Triple-	Count	3	19	22
		% within Groups	13.6%	86.4%	100.0%
Total		Count	20	72	92
		% within Groups	21.7%	78.3%	100.0%
	Value	*P*			
Pearson χ^2	5.668[a]	.129			

[a] Two cells (25.0%) have expected count less than 5. The minimum expected count is 3.26.

Table 5
There is not a significant correlation between tumor groups and multifocality, $P = .647$ (>.05).

			Multifocality		Total
			(−)	(+)	
Groups	Luminal A	Count	13	10	23
		% within groups	56.5%	43.5%	100.0%
	Luminal B	Count	23	9	32
		% within groups	71.9%	28.1%	100.0%
	HER-2 (+)	Count	9	6	15
		% within groups	60.0%	40.0%	100.0%
	Triple (−)	Count	15	7	22
		% within groups	68.2%	31.8%	100.0%
Total		Count	60	32	92
		% within groups	65.2%	34.8%	100.0%
	Value	*df*	*P*		
Pearson χ^2	1.657[a]	3	.647		

[a] Zero cells (0.0%) have expected count of less than 5. The minimum expected count is 5.22.

There was not a significant difference between groups in terms of patient age, tumor type, and multifocality (Tables 3-5). Conversely, a significant difference between groups in terms of tumor grades and lymphovascular invasion ($P = .042$) was established (Tables 6, 7).

When approached in an individual basis:

> In luminal A group, p53 expression was significantly high in grade III tumors (Table 8). In p53-negative ones, tumor types other than invasive ductal carcinoma (IDC) was remarkable with 47.4% ratio. There was not a significant difference in terms of patient age, multifocality, and lymphovascular invasion. MDM2 amplification was seen only in 1 case (4.3%) in luminal A group.

Table 6
There is a significant correlation between tumor groups and tumor grade, $P = .000$ (<.05).

			Grade		Total
			Low	High	
Groups	Luminal A	Count	21	2	23
		% within groups	91.3%	8.7%	100.0%
	Luminal B	Count	12	20	32
		% within groups	37.5%	62.5%	100.0%
	HER-2	Count	2	13	15
		% within groups	13.3%	86.7%	100.0%
	Triple (−)	Count	5	17	22
		% within groups	22.7%	77.3%	100.0%
Total		Count	40	52	92
		% within groups	43.5%	56.5%	100.0%
	Value	*P*			
Pearson χ^2	31.275[a]	.000			

[a] Zero cells (0.0%) have expected count of less than 5. The minimum expected count is 6.52.

Table 7
There is a significant correlation between tumor groups and lymphovascular invasion, $P = .042$ (<.05).

			LVI		Total
			(−)	(+)	
Groups	Luminal A	Count	17	6	23
		% within groups	73.9%	26.1%	100.0%
	Luminal B	Count	22	10	32
		% within groups	68.8%	31.3%	100.0%
	HER-2	Count	5	10	15
		% within groups	33.3%	66.7%	100.0%
	Triple (−)	Count	16	6	22
		% within groups	72.7%	27.3%	100.0%
Total		Count	60	32	92
		% within groups	65.2%	34.8%	100.0%
	Value	*P*			
Pearson χ^2	8.212[a]	.042			

Abbreviation: LVI, lymphovascular invasion.
[a] Zero cells (0.0%) have expected count of less than 5. The minimum expected count is 5.22.

Table 8
p53 is positive especially in high-grade tumors of luminal A group, $P = .002$, and IDC excluded tumor types are relatively high in p53-negative tumors, $P = .034$ (≤.05).

Luminal A	p53−	p53+	*P*
Age			
<50	5 (26.3%)	1 (25%)	.957
>50	14 (73.7%)	3 (75%)	
Multifocality			
(−)	11 (57.9%)	2 (50%)	.772
(+)	8 (42.1%)	2 (50%)	
Grade			
Low	19 (100%)	2 (50%)	.002
High	0 (0%)	2 (50%)	
LVI			
(−)	15 (78.9%)	2 (50%)	.231
(+)	4 (21.1%)	2 (50%)	
Tumor type			
IDC	10 (52.6%)	4 (100%)	.034
Other	9 (47.4%)	0 (0%)	

In luminal B group, MDM2 amplification was seen in 3 cases (9.4%). There was not a significant relationship between p53 and other parameters (Table 9).
In HER-2 group, MDM2 amplification was not seen. There was not a correlation between p53 and other parameters (Table 10).
In triple-negative group, MDM2 amplification was seen only in 1 case (4.5%). There was an inverse correlation between p53 and multifocality. There was not a significant relationship between p53 and other parameters (Table 11).

Table 9
There is not a significant correlation between p53 and other parameters in luminal B tumors.

Luminal B	p53−	p53+	*P*
Age			
<50	12 (54.5%)	4 (40%)	.582
>50	10 (45.5%)	6 (60%)	
Multifocality			
(−)	14 (63.6%)	9 (90%)	.124
(+)	8 (36.4%)	1 (10%)	
Grade			
Low	10 (45.5%)	2 (20%)	.168
High	12 (54.5%)	8 (80%)	
LVI			
(−)	13 (59.1%)	9 (90%)	.080
(+)	9 (49.9%)	1 (10%)	
Tumor type			
IDC	19 (86.4%)	8 (80%)	.646
Other	3 (13.6%)	2 (20%)	

Table 10
There is not a significant correlation between p53 and other parameters in HER-2 (+) tumors.

HER-2	p53−	p53+	*P*
Age			
<50	2 (25%)	3 (42.9%)	.464
>50	6 (75%)	4 (57.1%)	
Multifocality			
(−)	4 (50%)	5 (71.40%)	.398
(+)	4 (50%)	2 (28.6%)	
Grade			
Low	2 (25%)	0 (0%)	.155
High	6 (75%)	7 (100%)	
LVI			
(−)	2 (25%)	3 (42.9%)	.464
(+)	6 (75%)	4 (57.1%)	
Tumor type			
IDC	6 (75%)	6 (85.7%)	.605
Other	2 (25%)	1 (14.3%)	

4. Discussion

For many decades, conventional breast cancer classification systems were only based on the histologic appearances of breast cancers, although histologic subtypes have a limited impact on therapeutic decision making. In fact, clinicopathologic parameters such histologic grade; lymph node metastasis; lymphovascular invasion; and predictive biomarkers such as ER, PR, and HER-2 status have more impact on prognosis [16].

Over the past decade, microarray-based gene expression studies showed that breast cancers have different distinct molecular features. Four different breast cancer subtypes were identified: basal like, HER-2 enriched, luminal, and normal breast like. Subsequently, luminal group was divided into luminal A and luminal B groups. Furthermore, studies with larger data sets resulted in discovery of additional groups: interferon-rich, claudin-low, and molecular apocrine types [16].

The researchers have defined 50 genes to identify 4 major intrinsic subtypes. Luminal breast cancers have ER-associated genes and can be divided into 2 subtypes according to the proliferation-associated gene expression index (MKI67/Ki-67). Ki-67 cutoff was established as 14% by comparing gene expression profile and IHC data. This cutoff has a sensitivity of 72% and a specificity of 77% to distinguish luminal A from luminal B breast cancers. The subtypes as defined by gene expression profile were included in the 2011 St Gallen International Expert Consensus [16]. Immunohistochemical definition of molecular subtypes is accepted as the most appropriate classification for today's daily practice [16]. Afterwards, the current classification undergoes revision at the 2013 and 2015 St Gallen International Breast Cancer conferences [17-19].

Table 11
There is not a significant correlation between p53 and other parameters in triple (−) tumors.

Triple−	p53−	p53+	*P*
Age			
<50	5 (55.6%)	6 (46.2%)	.665
>50	4 (44.4%)	7 (53.8%)	
Multifocality			
(−)	4 (44.4%)	11 (84.6%)	.329
(+)	5 (55.6%)	2 (15.4%)	
Grade			
Low	3 (33.3%)	0 (0%)	.155
High	6 (66.7%)	2 (15.4%)	
LVI			
(−)	3 (25%)	11 (84.6%)	.323
(+)	6 (75%)	4 (57.1%)	
Tumor type			
IDC	7 (77.8%)	12 (92.3%)	.323
Other	2 (22.2%)	1 (7.7%)	

Although Ki-67 index is 14% in 2011 St Gallen Consensus, it was accepted as greater than or equal to 20% in 2013 St Gallen Consensus. In 14th St Gallen Breast Cancer Conference, 2015, this ratio (14%) has been found insufficient in the definition of 1 of 5 cases in luminal B breast cancer, and the Ki-67 index was accepted as greater than or equal to 20% [17-22].

In this present study, the classification of breast cancer cases was based on 2012 World Health Organization classification [2]. Molecular subtypes were based on IHC procedures as luminal A, luminal B, HER-2 (+), and triple negative. The Ki-67 index is determined as greater than or equal to 20% to distinguish luminal A from luminal B breast cancers according to the 2013 and 2015 St Gallen Consensus.

Ki-67 is a nuclear antigen present during all active phases of the cell cycle (G1, S, G2, and mitosis), except resting cells (G0).

p53 is a tumor suppressor protein that has a key role in various cellular functions: cell cycle, apoptosis, DNA repair, angiogenesis, senescence, cellular metabolism, and humoral immunity [23-26]. Correlated with the tumor suppressor function of p53, more than 50% of human tumors contain a mutation or deletion of the p53 gene. In cancers with wild-type p53, the function of p53 is down-regulated by MDM2 oncoprotein by various mechanisms: directly inhibiting the transcriptional function of p53, nuclear-cytoplasmic transportation of p53, and the proteasomal degradation of p53 by E3 ubiquitin ligase activity [23,24].

MDM2 is also activated by p53 itself; therefore, the inhibition of the MDM2-p53 interaction leads to the malignant transformation [24]. Design of nonpeptide, small molecule inhibitors that block the MDM2-p53 interaction has been sought as an attractive strategy to activate p53 for the treatment of cancer [26-29].

The tumor suppressor gene p53 is often inactivated in breast cancer cells due to gene mutation or overexpression of its repressors. Target therapies for inhibitors of p53 could lead to tumor suppression by restoration of p53 activity, and such an approach is a promising strategy for future control of breast cancer [28].

MDM2 is modified at transcriptional, posttranscriptional, and posttranslational levels to control p53 activity [30]. This modification is different in normal and stressed cells. Errors in these regulatory mechanisms can result in aberrant MDM2 expression and failure to initiate programmed cell death in response to DNA damage [30]. Such errors at the MDM2 locus and changes in posttranscriptional and posttranslational regulation of MDM2 may have severe consequences as evidenced by tumor phenotypes [30].

In a research with a total of 4000 cases, MDM2 amplification rate has been estimated 6% to 7% in 28 different human carcinomas [26].

MDM2-mediated p53 inhibition is well known, but MDM2 also interacts with many additional proteins such as PTEN, NF-kB, Fli-1, Raf, SMAD3/4, ataxia telangiectasia mutated (ATM), AKT, cyclin G, and MDM4 [30].

MDM2 has p53-independent oncogenic effects. MDM2 expression led to a decrease in E-cadherin levels and a subsequent increase in cell motility in breast carcinoma [12].

In response to ionizing radiation, p53 is phosphorylated by the ATM kinase, which inhibits MDM2 binding; however, after exposure to ultraviolet radiation, p53 is modified by the related ATM and Rad3-related kinase [12].

The p53 protein accumulates at times of cellular or genotoxic stress. p53 functions primarily as a transcription factor to promote cell cycle arrest and DNA repair, to initiate and maintain a senescent phenotype, or to promote apoptosis [12]. p53 functions to eliminate and inhibit the proliferation of abnormal cells, thereby preventing neoplastic development. p53 contributes to maintenance of G2 cell cycle arrest over cyclin-dependent kinase inhibitor $p21^{Waf1}$, 14-3-3σ, and cyclin B1–CDK1 complexes [3]. Wild-type p53 ribonucleotide reductase gene, p53R2, directly activates a number of genes that function in pathways of DNA repair [3]. p53 directly induces proapoptotic proteins over apoptosis regulators such as Apaf1, PUMA, p53AIP1, PIDD and NOXA, and lead apoptosis [3]. p53 induces maspin, a serine protease inhibitor that inhibits angiogenesis, invasion, and metastasis [3]. A potential defect in those functions results in the failure in the elimination of the damaged cells and apoptosis leading to the cancer formation.

One of the most commonly deleted chromosomal regions in breast cancer is 11q23-q25, which contains a number of putative tumor suppressor loci, including ATM, CHK1, PPP2R1B, and PIG8. A recent study determined that the gene most frequently mutated in this region was PIG8, a gene induced by p53 and a putative mediator of p53-dependent apoptosis. Loss of PIG8 function via inactivating mutations may be a potential mechanism of p53 dysfunction in breast cancer [3]. The MDM2 protooncogene is overexpressed and amplified in a variety of human tumors [31]. The tumorigenic activity of MDM2 is due to its ability to target p53 for degradation, leading to inhibition of p53-induced cell growth arrest and apoptosis [31]. Reactivation of p53 in the approximately 50% of tumors that retain a functional p53 has served as potential approach in the development of cancer therapy [32]. By targeting MDM2 in cancers with wild-type p53, it may be possible to restore p53 function to control tumor growth [32]. The potential of inhibitors of MDM2 E3 ligase is a new class of anticancer drugs [32].

5. Conclusion

p53 expression is relatively low in luminal-type breast cancers in comparison with HER-2 and triple-negative subtypes. Besides, in luminal A group, p53 expression is seen especially in the grade III breast cancers. It can be concluded that MDM2 gene amplification has not a significant role in breast cancer pathogenesis, but regarding another mechanisms affecting MDM2 gene and MDM2 protein, which interfere with p53 gene functions, further studies need to be done.

Relatively less number of HER-2 (+) subtype among all other molecular subtypes lead to the statistically impairment, which is the constraint of this study.

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