Immunohistochemical detection of P53 and Mdm2 in vitiligo

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ABSTRACT

Background: Vitiligo is a common depigmented skin disorder that is caused by selective destruction of melanocytes. It is generally accepted that the main function of melanin resides in the protection of skin cells against the deleterious effect of ultraviolet rays (UVRs). Association of vitiligo and skin cancer has been a subject of controversy. Occurrence of skin cancer in long-lasting vitiligo is rare despite multiple evidences of DNA damage in vitiliginous skin. **Aim:** To detect the expression of P53 and Mdm2 proteins in both depigmented and normally pigmented skin of vitiligo patients and to compare it to control subjects suffering from nonmelanoma skin cancer (NMSC). **Materials and Methods:** Thirty-four patients with vitiligo and 30 age and sex-matched patients with nodulo-ulcerative basal cell carcinoma (BCC) as a control group were selected. Both patients and control subjects had outdoor occupations. Skin biopsies were taken from each case and control subjects. Histopathological examination of Hematoxylin and eosin-stained sections was done. Expression of P53 and Mdm2 proteins were examined immunohistochemically. **Results:** Both P53 and Mdm2 were strongly expressed in depigmented as well as normally pigmented skin of vitiligo patients. This expression involved the epidermis, skin adnexa and blood vessels with significant differences between cases and controls. **Conclusions:** The overexpression of P53 and Mdm2 proteins in both normally pigmented and depigmented skin of patients with vitiligo could contribute to the decreased occurrence of actinic damage and NMSC in these patients.

Key words: Mdm2, P53, vitiligo

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INTRODUCTION

Vitiligo is an acquired pigmentary disorder characterized by the presence of well-circumscribed depigmented milky white macules and patches[1] resulting from loss of melanocytes from epidermis, mucous membrane, inner ears, eyes, hair and occasionally from the hair bulbs.[2]

Numerous epidemiological studies have documented that people with fair skin, as well as affected individuals with albinism are significantly more sun sensitive compared with darker skin races.[3] Furthermore, these groups experience multiple sunburns during their life time together with high risk of development of actinic skin damage and skin cancer.[4]

Patients with vitiligo are expected to suffer from more melanoma and nonmelanoma skin cancer (NMSC) than the general population due to lack of melanin. However, there is no significantly increased risk of these cancers even in long-standing disease.[5]

P53 is considered to be the cellular gatekeeper for growth and division. The P53 gene product is a nuclear protein (wild type P53 protein) that binds to specific DNA sequences and functions as a transcription factor. It regulates the expression of genes that control cell cycle progression, induction of apoptosis, DNA repair and regulates functions involved in cellular responses to stress. These functions occur through mediating arrest of mammalian cells at one of two major cell cycle checkpoints, in G1 near the border of S phase or in G2 before mitosis. P53 modulates DNA repair processes through the arrest of cell cycle progression that may provide time for the repair of DNA damage. However, in some circumstances of irreversible damage, stress signaling will initiate P53 dependent apoptosis thus preventing the fixation of DNA damage as mutations.[6]

In dividing cells, the central regulator of P53 function is the oncogene Mdm2 which binds to and regulates the activity of the P53 protein. It is a ubiquitin ligase which mediates ubiquitination

of P53, thereby targeting it for degradation. In this way, P53 levels are kept low in normal cells.[7]

The aim of this work was to detect the degree of expression of P53 and Mdm2 proteins in both depigmented as well as normally pigmented skin of vitiligo patients and to compare that to control subjects suffering from NMSC.

MATERIALS AND METHODS

Our study is a case-control study. It included 34 patients with vitiligo (localized and generalized) and 30 age and sex-matched patients with nodulo-ulcerative BCC (representative of NMSC) as a control group. Both cases and control have outdoor occupations with excessive sun exposure and were selected randomly from Dermatology outpatient clinic, Menoufiya University Hospital, Menoufiya Governorate, Egypt during the period between January to October 2010. Cases and control subjects had skin phototypes 3 and 4. The local Ethics Committee approved the study.

Each of the selected cases after taking consent was subjected to complete history-taking, complete general and dermatologic examination and skin biopsy from depigmented as well as normally pigmented UVR-exposed skin. Biopsies were taken from control subjects (from normal perilesional skin) after consent.

Examination of hematoxylin and eosin-stained sections was done to evaluate and verify epidermal and dermal pathological alterations.

Immunohistochemical staining was done using:

1) Mouse monocolonal anti-P53 antibody (1:50) (Catalogue no. # MS–738-R7-LabVision/ Neomarkers-USA)
2) Rabbit polyclonal anti-Mdm2 antibody (1:50) (Catalogue no. #RB-9218- LabVision/ Neomarkers-USA)

Immunostaining interpretation:
Positive immunostaining was considered when >5% of epidermal, dermal or adnexal cells showed brown nuclear staining.[8]

P53 and Mdm2 expression were evaluated by using Quickscore, which considers both the intensity of staining and percentage of positive cells in a semiquantitative pattern according to the following formula:

The intensity of immunostaining (I) was scored as 0: negative, 1: weak, 2: moderate and 3: strong.

The percentage of positive cells (P) scored as 1: 0-4%, 2: 5-19%, 3: 20-39%, 4: 40-59%, 5: 60-79% and 6: 80-100%.

Quickscore was then calculated by multiplying the intensity by the percentage of positive cells (I X P) with a final score ranged from 0 to 18 where more than 10 was assigned as strong, 6-10: moderate, 1-5: weak and 0: negative.[9]

Statistical analysis

Data were collected, tabulated and statistically analyzed using "Statistical Package for the Social Sciences (SPSS) version 11" program. χ^2 and Fisher's exact tests were used to compare qualitative data. Differences were considered statistically significant when $P<0.05$ and highly significant when $P<0.001$.[10]

RESULTS

Table 1 summarizes the clinical and histopathological data of the studied patients.

Immunohistochemical staining

a) P53 (according to intensity of immunostaining)

Control subjects

Immunoreactivity ranged from mild to strong [Table 2].

Vitiligo patients

Depigmented skin

The epidermis showed strong positive nuclear immunostaining in the majority of cases and moderate immunostaining in few cases [Figure 1a, Table 2]. In the dermis, skin appendages

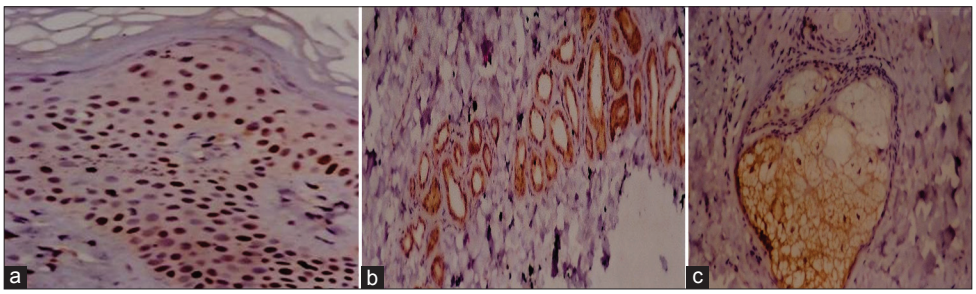

Figure 1: P53 expression in depigmented and normally pigmented patients' skin. (a) strong P53 immunestaining in basal and suprabasal layers in depigmented skin [Immunoperoxidase, ×400]. (b) strong staining of sweat glands of depigmented skin [Immunoperoxidase, ×100]. (c) strong staining of sebaceous glands of depigmented skin [Immunoperoxidase, ×400].

Table 1: Clinical and histopathological data of studied patients

Variable	X± SD	No	%
Age	36±2 ys	-	-
9-57 yrs			
Duration	5 ±2 ys	-	-
8 months-34 yrs			
Sex			
Male		20	58.8
Female		14	41.2
Distribution of vitiligo lesions			
Generalized		18	52.9
Acrofacial		9	26.5
Focal		7	20.6
Associated diseases with vitiligo			
Occular manifestations		6	17.6
Acoustic manifestations		3	8.8
Diabetes mellitus		2	5.9
Alopecia areata		1	2.9
Family history of the disease			
Positive family history		8	23.5
Negative family history		26	76.5
Past history of melanoma or nonmelanoma skin cancer			
Negative		34	100.0
Positive		0	0.0
Thickness of the epidermis			
Atrophy		20	58.8
Normal		14	42.2
Structure of stratum cornium			
Basket weave		22	64.7
Mixed		4	11.8
Compact		8	23.5
Parakeratosis		8	23.6
Sun-burn cells		28	82.4
Presence of stratum granulosum		34	100
Basement membrane			
Absent		17	50
Focal thickening		17	50
Solar elastosis		4	11.5
Vaculated keratinocytes		14	41.2
Dermal papillae			
Normal		4	11.8
Atrophied		30	88.2
Inflammatory infiltrate			
Mild		13	38.2
Moderate		13	38.2
Excessive		8	23.6

X: Mean SD: Standard deviation

and endothelium of the blood vessels showed strong positive nuclear immunostaining [Figure 1b, c] [Table 2].

Normally pigmented skin

The epidermis showed mild to moderate nuclear and cytoplasmic immunostaining [Table 2] while the dermis showed moderate nuclear and cytoplasmic immunostaining of sweat glands, endothelium of the blood vessels, hair follicles and sebaceous glands [Table 2].

b) Mdm2 (according to intensity of immunostaining)

Control subjects

Mild immunoreactive nuclear staining in the epidermis and skin adenxa was found in all studied subjects [Table 3].

Vitiligo patients

Depigmented skin

Both epidermis and dermis showed strong nuclear immunostaining in most of the cases and moderate immunostaining in some cases [Table 3].

Normally pigmented skin

The epidermis showed moderate nuclear and cytoplasmic staining in most of the cases and mild staining in few cases [Table 3] while the dermis showed moderate staining of sweat glands, sebaceous glands, hair follicles and endothelium of the blood vessels in all cases [Table 3].

By applying Quickscore formula, positive P53 expression was moderate in 41.2% and strong in 58.8% of the studied cases. Regarding Mdm2 expression, Quickscore formula showed moderate staining in 53% of cases and strong in 47% of cases [Table 4].

DISCUSSION

Vitiligo is an acquired depigmentation disorder affecting 0.1-2% of the world population. This disease is characterized by the loss of the melanin pigment due to the partial or complete absence of functioning melanocytes in the affected areas.[11]

Vitiligo is associated with a number of systemic, autoimmune and cutaneous disorders. Vitiligo may precede these diseases which indicates the potential usefulness of screening of vitiligo patients.[1] In our study, ocular manifestations including myopia and nystagmus, acoustic problems, diabetes mellitus and alopecia areata were detected in examined patients.

Obtained results of histopathological examination of hematoxylin and eosin-stained sections were similar to studies of other authors.[5,12]

There is a plethora of evidence that the entire epidermis of patients with vitiligo shows multiple signs of oxidative stress, including the presence of allantoin,[13] massive amounts

Table 2: P53 expression in studied groups

	Cases				Control		χ^2	*P*-value
	Normal skin		Depigmented skin		No	%		
	No.	%	No.	%				
Epidermis								
Mild	6	17.7	-	-	21	70	127.1	< 0.001*
Moderate	28	82.3	4	12	6	20		
Strong	-	-	30	88	3	10		
Dermis								
Mild	-	-	-	-	21	70	127.1	< 0.001*
Moderate	31	91	9	26.5	6	20		
Strong	3	9	25	73.5	3	10		

χ^2: Chi square *: highly significant

Table 3: Mdm2 expression in studied groups

	Cases				Control		χ^2	*P*-value
	Normal skin		Depigmented skin		No.	%		
	No	%	No	%				
Epidermis								
Mild	4	12	-	-	30	100.0	156.0	<0.001*
Moderate	30	88	9	26.5	-	-		
Strong	-	-	25	73.5	-	-		
Dermis								
Mild	-	-	-	-	30	100.0	156.0	<0.001*
Moderate	34	100	7	20.5	-	-		
Strong	-	-	27	79.5	-	-		

χ^2: Chi square *: highly significant

of hydrogen peroxide (H_2O_2),[14] low catalase[15] and other important antioxidant enzymes, including thioredoxin reductase/ thioredoxin, glutathione peroxidase, glutathione reductase, superoxide dismutases and the repair enzymes methionine sulfoxide reductases A and B.[16]

In addition, there is systemic oxidative stress, including the presence of DNA damage in peripheral blood lymphocytes and alteration of mitochondria in blood mononuclear cells.[17]

In vitiligo, H_2O_2-mediated oxidation affects many proteins and peptides, yielding altered or even complete loss of functionality.[18]

This scenario leads to many consequences, including the loss of functioning melanocytes, lipid peroxidation with formation of vacuoles in epidermal melanocytes and keratinocytes.[1] Consequently, it was speculated that vitiligo patients are more vulnerable than normal population to photodamage, premature aging and NMSC.[19]

However, there is no significantly increased risk of actinic damage or NMSC even in long standing disease.[5] Even more surprising is that the skin of these individuals is significantly younger compared with age-matched healthy individuals.[18]

Therefore a question arises, which protective mechanism could be involved in the skin of these patients preventing the initiation of these cancers?[20]

Clearly under these conditions, DNA damage would be an expected must. In this context, it is noteworthy that this patient group exhibits persistent high levels of functioning wild type P53 protein in their skin.[18]

Schallreuter *et al*.[5] demonstrated that P53 protein markedly increases in the nuclei of the epidermal cells following UVR and H_2O_2 accumulation.[21] As accumulation of the genomic aberrations is the key carcinogenic mechanism, the rapid induction of P53 activity in response to genomic damage thus serves to ensure that cells carrying such damage are effectively taken care of.[22]

Mdm2 is a key player in regulation of P53. Mdm2 binds to P53 and leads to complete elimination of P53 through proteolytic degradation.[23]

There are few reports of NMSC in vitiligo skin. Lisi[24] stated that these cancers are considered anecdotal since so far, only a few cases have been reported in the literature.

Table 4: Correlation between Quickscore evaluation for P53 and Mdm2 expression and clinical data

	Quick score for P53				
	Moderate		Strong		*P*-value
	No.	%	No.	%	
Age					
<20	8	57.2	11	55	0.1
>20	6	42.8	9	45	
Sex					
Male	5	35.7	15	75	0.7
Female	9	64.3	5	25	
Type					
Generalized	7	50	11	55	0.03†
Acrofacial	3	21.4	6	30	
Focal	4	28.6	3	15	
	Quick score for Mdm2				
Age					
<20	7	38.8	9	56.25	0.23
>20	11	61.2	7	43.75	
Sex					
Male	9	50	11	68.75	0.096
Female	9	50	5	31.25	
Type					
Generalized	4	22.2	14	87.5	0.01†
Acrofacial	9	50	0	0.0	
Focal	5	27.8	2	12.5	

#:Fischer exact †:significant

Lassus *et al*.[25] reported two patients, one with SCC and another with BCC, but it is not known whether these tumors were located on vitiliginous areas or not. Saarinen *et al*.[26] reported a case of SCC and actinic keratoses in a patient with generalized vitiligo. The patient was given high doses of systemic corticosteroids for his chronic obstructive pulmonary disease. It remains uncertain whether the prolonged course of oral corticosteroids given to this patient sufficiently compromised his immune system to permit the development of premalignant and malignant skin lesions. It is widely accepted that there is an increased incidence of skin carcinomas in immunocompromised individuals.[27]

Park *et al*.[28] reported a case of SCC in vitiligo lesion after long-term PUVA therapy. Although PUVA has been used to treat vitiligo since 1976,[29] no skin cancers related to PUVA therapy in vitiligo patients were reported until 1996.[30] SCC after long-term PUVA therapy is much rarer in vitiligo patients than in psoriasis patients.[28]

The aim of this study was the immunohistochemical detection of P53 and Mdm2 in depigmented and normally pigmented skin of vitiligo patients, comparing them with patients with NMSC and correlating between their expression and different clinical parameters.

In this study, P53 protein expression in the epidermis was significantly higher in both normally pigmented and depigmented skin in vitiligo patient than control subjects. Our results are in agreement with Schalleurter *et al*.[8]

Moreover this study showed that the expression of P53 protein extended also to the skin adnexa such as sweat glands, sebaceous glands, hair follicles as well as to the endothelium of the blood vessels. To the best of our knowledge, such a phenomenon has not been reported in previous similar studies. However, this can be explained by accumulation of genomic aberrations due to DNA damage, and oxidative stress in adenxal cells which lead to rapid induction of P53.

Saarinen *et al*.[26] explained the rarity of cancer in vitiligo skin by the tendency of patients to avoid sun exposure, because it accentuates the contrast of skin color due to the lesions, and consequently, chronic sun exposure may decrease in vitiligo patients. However, in our study, all patients have outdoor occupations with chronic sun exposure.

P53 expression was significantly higher in cases of generalized vitiligo than cases of localized (focal and acrofacial) vitiligo. To the best of our knowledge, this finding has not been reported in previous similar studies. It can be explained by the longer duration of disease in generalized vitiligo than localized cases. It is established that the development of NMSC is merely a matter of disease duration and intensity sun light exposure.[28]

In this current study, Mdm2 expression was also significantly higher in vitiligo patients both in normally pigmented and depigmented skin than the control subjects. This is contrary to what was mentioned by Schalleurter *et al*.[8] who reported that Mdm2 protein remains unchanged in both depigmented and normally pigmented skin compared with control subjects. Chen *et al*.[31] suggested that increased expression of Mdm2 is the result of overexpression of P53 (because P53 binds specifically to the Mdm2 gene and stimulates its transcription leading to production of Mdm2 protein). Mdm2 protein binds to P53 and inactivates it. Interestingly, experimental evidence also indicates that Mdm2 overexpression causes G1 arrest in normal cells.[32] So the level of both proteins is elevated in the cell and in this case Mdm2 has a supportive role to P53 as it can stop the cell cycle in G1 phase.[33]

Mdm2 expression was significantly higher in cases of generalized vitiligo than cases of localized (focal and acrofacial) vitiligo. To the best of our knowledge, there was no comment on this phenomenon in the previous similar studies. As Mdm2 follows P53, the increased expression of P53 in generalized vitiligo as mentioned above could explain the increased Mdm2 expression.[31]

We conclude that vitiligo patients have protective effects against UVR-induced DNA damage. This could be due to

overexpression of P53 and Mdm2 proteins in their skin. Further studies are needed on larger numbers of patients with different skin phototypes and diverse environmental conditions.

REFERENCES

1. El Darouti MA, Marzouk SA, Azzam O, Fawzi MM, Abdel Halim MR, Zayed AA, *et al.* Vitiligo vs hypopigmented mycosis fungoides. Eur J Dermatol 2006;16:17-22.
2. Zhang XJ, Chen JJ, Liu JB. The genetic concept of vitiligo. J Dermatol Sci 2005;39:137-46.
3. Bam M, Bagchi T. Absence of tyrosinase-related protein-2/dopachrome tautomerase transcripts in PBMCs from vitiligo patients. Scand J Immunol 2009;69:366-73.
4. Salasch SK. Epidemiolgoy of actinic keratosis and squamous cell carcinoma. J Am Acad Dermatol 2000;42:54-7.
5. Schallreuter KU, Tobin DJ, Panske A. Decreased photodamage and low incidence of non-melanoma skin cancer in 136 sun exposed Caucasian patients with vitiligo. Dermatol 2002;204:194-201.
6. Appella E, Anderson CW. Post-translational modifications and activation of p53 by genotoxic stresses. Eur J Biochem 2001;268:2764-8.
7. Zhang XP, Liu F, Wang W. Two-phase dynamics of p53 in the DNA damage response. Proc Natl Acad Sci U S A 2011;108:8990-5.
8. Schallreuter KU, Behrens-Williams S, Khaliq TP, Picksley SM, Peters EM, Marles LK, *et al*. Increased epidermal functioning wild type p53 expression in vitiligo. Exp Dermatol 2003;12:268-77.
9. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The Mdm2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 1992;69:1237-45.
10. Alain D. The Politics of Large Numbers: A History of Statistical Reasoning. 2nd ed. Trans. Camille Naish. Paris. Harvard University Press; 2004.
11. Lv Y, Li Q, Wang L, Gao T. Use of anti-tumor necrosis factor agents: a possible therapy for vitiligo. Med hypotheses 2009;725:546-7.
12. Tobin DJ, Swanson NN, Pitteknow MR, Peters EM, Schallreuter KU. Melanocytes are not absent in lesional skin of long duration vitiligo. J Pathol 2000;191:407-16.
13. Shalbaf M, Gibbons NC, Wood JM, Maitland DJ, Rokos H, Elwary SM, *et al*. Presence of epidermal allantoin further supports oxidative stress in vitiligo. Exp Dermatol 2008;17:761-70.
14. Schallreuter KU, Moore J, Wood JM, Beazley WD, Gaze DC, Tobin DJ, *et al*. *In vivo* and *in vitro* evidence for hydrogen peroxide (H2O2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase. J Investig Dermatol Symp Proc 1999;4:91-6.
15. Schallreuter KU, Wood JM, Berger J. Low catalase levels in the epidermis of patients with vitiligo. J Invest Dermatol 1991;97:1081-5.
16. Hasse S, Gibbons NC, Rokos H, Marles LK, Schallreuter KU. Perturbed 6-tetrahydrobiopterin recycling via decreased dihydropteridine reductase in vitiligo: More evidence for H2O2 stress. J Invest Dermatol 2004;122:307-13.
17. Schallreuter KU, Bahadoran P, Picardo M, Slominski A, Elassiuty YE, Kemp EH, *et al.* Vitiligo pathogenesis: Autoimmune disease, genetic defect, excessive reactive oxygen species, calcium imbalance, or what else? Exp Dermatol 2008;17:139-60.
18. Wood JM, Decker H, Hartmann H, Chavan B, Rokos H, Spencer JD, *et al*. Senile hair graying: H2O2- mediated oxidative stress affects human hair color by blunting methionine sulfoxide repair. FASEB J 2009;23:2065-75.
19. Westerhof W, Schallreuter KU. PUVA for vitiligo and skin cancer. Clin Exp Dermatol 1997;22:54-9.
20. Hartmann A, Bedenk C, Keikavoussi P, Becker JC, Hamm H, Bröcker EB. Vitiligo and melanoma-associated hypopigmentation (MAH): Shared and discriminative features. J Dtsch Dermatol Ges 2008;6:1053-9.
21. Prather RS, Boquest AC, Day BN. Cell cycle analysis of cultured porcine Mammary Cells. Cloning 1999;1:17-24.
22. Brenkman AB, Dekeizer PL, Jochemsen AG, Burgering BM. Mdm2 induced mono-ubiquitination of Foxo4. PloS One 2008;3:2819-24.
23. Duan W, Gao L, Wu X, Zhang Y, Otterson GA, Villalona-Calero MA. Differential response between the p53 ubiquitin-protein ligases and Mdm2 following DNA damage in human cancer cells. Exp Cell Res 2006;312:3370-78.
24. Lisi P. Vitiligine e cancro cutaneo. G Ital Dermatol 1972; 11: 427-29.
25. Lassus A, Apajalahti A, Blomqvist K, Mustakallio M, Kiistala U. Vitiligo and neoplasms. Acta Derm Venereol 1972;52:229-32.
26. Saarinen K, Lestringant GG, Masouye I, Frossard PM. Actinic damage and squamous cell carcinoma in sun-exposed skin affected by vitiligo. Br J Dermatol 2000;143:219-21.
27. McGregor JM, Proby CM. Skin cancer in transplant recipients. Lancet 1995;346:964-65.
28. Park HS, Lee YS, Chun DK. Squamous cell carcinoma in vitiligo lesion after long-term PUVA Therapy. J Eur Acad Dermatol Venereol 2003;17:578-80.
29. Parrish JA, Fitzpatrick TB, Shea C, Pathak MA. Photochemotherapy of vitiligo. Use of orally administered psoralens and a high-intensity long-wave ultraviolet light system. Arch Dermatol 1976;112:1531-4.
30. Buckley DA, Rogers S. Multiple keratoses and squamous carcinoma after PUVA treatment of vitiligo. Clin Exp Dermatol 1996;21:43-5.
31. Chen D, Zhan J, Li M, Rayburn ER, Wang H, Zhang R. RYBP stabilizes P53 by modulating Mdm2. EMBO Res 2009;10:166-72.
32. Moll UM, Petrenko O. The Mdm2-P53 interaction. Mol Cancer Res 2003;1:1001-8.
33. Zhou BP, Liao Y, Xia W, Zou Y, Spohn B, Hung MC. HER-2/neu inducers ubiquitination via AKt-mediated Mdm2 phosphorylation. Nat Cell Biol 2001;3:973-82.

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