The proteasome subunit PSMA7 located on the 20q13 amplicon is overexpressed and associated with liver metastasis in colorectal cancer

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Abstract. The proteasome subunit PSMA7 located on the 20q13 amplicon was found to be differentially expressed in colorectal cancer by semiquantitative RT-PCR. PSMA7 mRNA was overexpressed in 37.5% (12/32) colorectal cancer tissues while it was either of a low level or not expressed in matched normal mucosa. The aim of this study was to examine the PSMA7 protein expression in 62 colorectal cancer primary sites, 34 lymph node metastatic sites and 13 liver metastatic sites by immunohistochemistry and clarify the correlations of this expression with the clinicopathological parameters. PSMA7 high expression was detected in 38.8% (24/62) colorectal cancer primary sites, 52.9% (18/34) lymph node metastatic sites and 100% (13/13) liver metastatic sites but not in the normal colorectal tissues. The PSMA7 high expression was significantly correlated with liver metastasis (P=0.028). Survival was significantly lower in patients with a PSMA7 high expression than in those with a PSMA7 low expression (P=0.0012). Moreover, in multivariate analysis, the PSMA7 expression demonstrated an independent prognostic factor (P=0.004, relative risk 5.057; 95% confidence interval, 1.682-15.201). These results indicated that PSMA7 may play an important role in colorectal cancer progression and provide a unique target site for the development of therapeutic drugs. The evaluation of PSMA7 expression in primary colorectal cancer at the time of surgery may be a valuable tool for defining patients with a high risk of developing liver metastasis.

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Introduction

Colorectal cancer (CRC) is one of the most common causes of cancer-related deaths throughout the world. Though in recent years progress has been made in the treatment of colorectal cancer, the metastatic potential of tumors is the major obstacle to successful treatment and the major cause of mortality in this type of malignancy (1). The majority of CRC patients die because of metastasis to the liver.

Molecular investigations have provided evidence that multiple genetic alterations are involved in colorectal tumorigenesis. There is considerable scientific and clinical interest in locating tumor stage-dependent chromosomal regions to find genes that are responsible for tumor progression and using such hotspots as predictive biomarkers for a pretherapeutic molecular staging and individual risk estimation. Karyotypic/cytogenetic data regarding CRC have been accumulated over the last 10 years and a gain of the long arm of chromosome 20 has been described as a common genetic aberration (2-4). The major finding of the present study was the significantly high mean relative copy number of chromosome 20q13 in CRCs with metastasis as compared with that in CRCs without metastasis (5).

These data suggest that the chromosome segment 20q13 harbors one or more oncogenes that are important for colorectal cancer development. Recently, the novel zinc finger gene *ZNF217*, which is amplified in CRC, was found on 20q13. However, the candidate genes that are clearly associated with the metastatic potential of CRC in this region (20q13 amplicon) remain unknown. In this study, we found that the proteasome subunit PSMA7 located on the 20q13 amplicon was overexpressed by semiquantitative RT-PCR in CRCs. We therefore examined PSMA7 protein expression in colorectal cancer by immunohistochemistry and clarified the correlations of its expression with the clinicopathological parameters.

Materials and methods

Patients and tissue samples. A total of 62 patients with colorectal adenocarcinoma who underwent surgery from

August 1999 to March 2005 at the Sir Run Run Shaw Hospital (Hangzhou, Zhejiang, P.R. China) were investigated in this study. There were 35 male and 27 female patients ranging in age from 24 to 79 years (59.50±13.31). Patients who received preoperative chemotherapy were excluded from this study. Thirty-four patients had lymph node metastases and 24 patients had liver metastases. Paraffin and frozen tumor and matched normal mucosa blocks were retrieved from 62 primary site, 34 lymph node metastatic site and 13 available liver metastatic site surgical specimens. All but 5 patients were available to be followed up for a period of 79 months or until death. The median follow-up period was 32.23 months (range, 4.60-70.40). Twenty patients died of colorectal cancer during the follow-up period. This study was approved and monitored by the ethics committee of Sir Run Run Shaw Hospital.

Semiquantitative RT-PCR. The extraction of total RNA from frozen sections of CRC specimens was carried out using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's specification. First-strand complementary DNA (cDNA) synthesis was performed using a GeneAmp RNA PCR core kit (Perkin Elmer, Branchburg, NJ). Briefly, mRNA from 2 μg of total RNA was reverse transcribed to cDNA in a 20 μl reaction volume containing 1X PCR buffer, 5 mM $MgCl_2$, 1 mM each of dNTP, 1 U/μl of RNase inhibitor, 2.5 U/μl MuLV enzyme and 2.5 mM Oligo-$d(T)_{16}$ or random hexamer primer. The RNA was first denatured at 95˚C for 10 min before adding it to the reaction mixture. cDNA was synthesized at 42˚C for 1 h followed by 95˚C for 10 min and stored at -20˚C until use.

PCR was performed to amplify PSMA7 in 32 cases (RNA quality of these cases was excellent and the amount of cDNA was normalized) with the following primers (Genbank Accession Number NM_002792): forward: 5'-TCA GTC AGG TGG CAA AAA CA-3' and reverse: 5'-ATG GAA AGG CCT ACA CAT CG-3' resulting in a DNA product of 227 bp. PCR conditions were 94˚C for 1 min, 52˚C for 30 sec and 72˚C for 30 sec, for 33 cycles, with initial denaturation at 95˚C for 5 min and a final elongation at 72˚C for 5 min. To normalize the amount of cDNA of each sample analyzed in semiquantitative RT-PCR, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene products were also amplified as an endogenous control of the expression. GAPDH forward primer: 5'-ACC ACA GTC CAT GCC ATC AC-3' and reverse primer: 5'-TCC ACC ACC CTG TTG CTG CC-3' resulting in a DNA product of 452 bp. Each cDNA sample was analyzed in triplicate with the appropriate negative controls. All PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide.

Immunohistochemistry. The ChemMate™ EnVision™ detection kit (Dako, Carpinteria, CA, USA) was used for immunohistochemistry (IHC) according to the company's recommended procedure. Briefly, after being deparaffinized and hydrated, the paraffin-embedded sections were placed in 0.01 M sodium citrate buffer (pH 6.0) and subjected to pressure cooker treatment for 2 min at full pressure with a domestic pressure cooker. After cooling to room temperature, the slides were rinsed with TBS (0.05 M Tris/0.15 M NaCl, pH 7.6). The endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide. The primary antibody used in this study is a mouse monoclonal antibody against the human PSMA7 protein (clone 1A10-3G12, Abnova, Taipei, Taiwan). The sections were incubated with the primary antibody (1:50 dilution) overnight at 4˚C. Then the ChemMate EnVision/HRP, Rabbit/Mouse (ENV) reagent was applied to the sections, followed by application of ChemMate DAB + Chromogen included in the kit. The slides were lightly counterstained with hematoxylin. For immunostaining of the negative controls, Dako antibody diluent was used in place of the primary antibody.

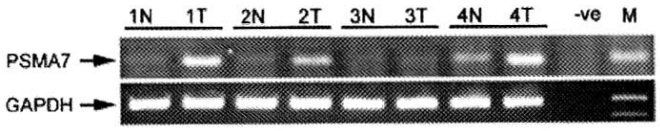

Figure 1. Representative semiquantitative RT-PCR results of PSMA7 in colorectal cancer tissues (T) and their matched normal mucosa (N). The expression of GAPDH served as a control; M, marker and -ve, negative control.

Evaluation of the PSMA7 protein expression level. The PSMA7 protein expression level in colorectal cancer cells was scored based on the intensity of cytoplasmic staining by a 4-point system: 0, negative; 1, weakly positive; 2, positive and 3, strongly positive. To examine the association of the PSMA7 expression level with the clinicopathological features, the patients were divided into two groups: PSMA7 low (0 and 1) or PSMA7 high (2 and 3). Immunostaining was scored independently on separate occasions by two investigators (Jin M and Liang YQ), who were blinded to the clinical information of the patients.

Statistical analysis. Statistical analysis was performed in SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). The two-tailed Chi-square and Fisher's exact test were used to analyze the association of the PSMA7 protein expression with the different clinicopathological parameters. Overall survival (OS) curves were estimated using the Kaplan-Meier method and any differences in the survival curves were compared by the log-rank test. For multivariate analysis, the Cox regression was used. For all tests, $P \leq 0.05$ was considered to be of statistical significance and 95% confidence intervals (95% CI) were used throughout.

Results

Identification of PSMA7 mRNA differentially expressed in colorectal cancer versus matched normal mucosa. To search for the PSMA7 mRNA expression status in colorectal cancer, semiquantitative RT-PCR was performed in 32 CRC cases (RNA quality of these cases was excellent and the amount of cDNA was normalized). PSMA7 mRNA was overexpressed in 37.5% (12/32) colorectal cancer tissues while PSMA7 mRNA was either of a low level or not expressed in matched normal mucosa. Representative semiquantitative RT-PCR results are shown in Fig. 1.

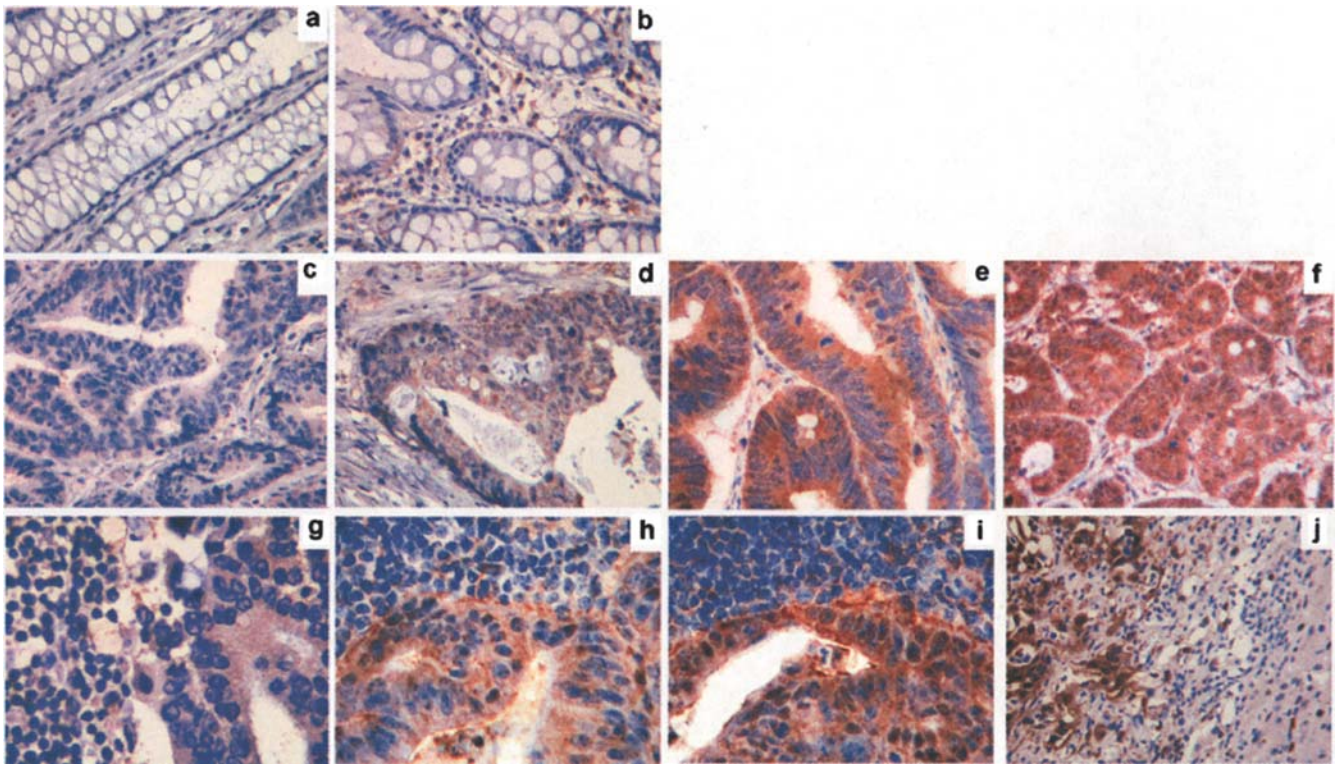

Figure 2. Representative PSMA7 immunohistochemical analysis results in the cytoplasm of non-tumorous, neoplastic and metastatic colon tissues. Non-tumorous colorectal epithelium was negative (a) or weakly positive (b). PSMA7-low (c and d) or PSMA7-high (e and f) expression in primary colorectal cancer cells. PSMA7-low (g) or PSMA7-high (h and i) expression in the colorectal cancer cell metastasis to lymph nodes. PSMA7-high (j) expression in the colorectal cancer cell metastasis to liver; original magnification, x400.

Correlation of the PSMA7 protein high expression with colorectal cancer liver metastasis. To ascertain the differential expression of PSMA7 in colorectal cancer, immunohistochemistry was performed in 62 colorectal cancer primary sites as well as 34 lymph node and 13 liver metastatic sites. The specimens evaluated for PSMA7 staining included normal colorectal mucosa near the neoplasms and at the surgical margins. PSMA7 protein was found to be expressed in the cytoplasm of colorectal epithelial cells and some stromal cells. PSMA7 protein high expression was detected in 38.8% (24/62) primary sites, 52.9% (18/34) lymph node metastatic sites and 100% (13/13) liver metastatic sites. The representative IHC results are shown in Fig. 2.

The correlations between the clinicopathological parameters of patients with colorectal cancer and the PSMA7 protein expression are summarized in Table I. The PSMA7 protein high expression was significantly correlated with liver metastasis (P=0.028) and Dukes' stage (A+B+C vs. D, P=0.034) according to the PSMA7 protein expression in the primary sites. When the patients were separated into 2 groups according to Dukes' stage as A+B vs. C+D, then PSMA7 expression had no significant association with Dukes' stage (P=0.068). This is consistent with the statistical results that PSMA7 protein high expression had no significant association with lymph node metastasis (P=0.795) since Dukes C represents the patients with locally advanced, lymph node-positive colorectal cancer. The PSMA7 protein high expression also had no significant correlation with patient gender, age, tumor location, differentiation or depth of invasion.

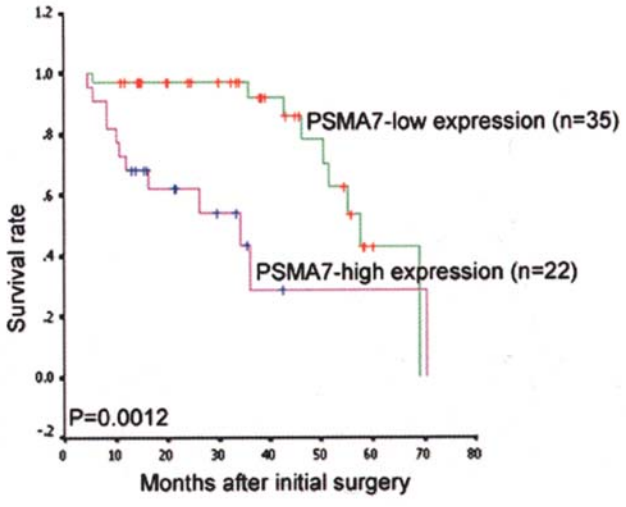

Figure 3. The survival curve of patients with colorectal cancer according to PSMA7 expression in the primary site. Survival of patients with PSMA7-high expression in the primay site was significantly worse than that of the patients with PSMA7-low expression (P=0.0012).

The PSMA7 protein high expression as an independent prognostic factor for colorectal cancer. The association of the PSMA7 protein expression and patient survival is shown in Fig. 3. The overall survival of patients with a PSMA7 high expression was significantly worse than that of the patients with a PSMA7 low expression according to the PSMA7

Table I. The association between PSMA7 immunohistochemical expression and clinicopathological parameters in colorectal cancer.

Clinicopathological parameters	N=62	PSMA7 expression		P-value
		Low (N=38)	High (N=24)	
Sex				
Male	35	21	14	1.000
Female	27	17	10	
Age				
≥59.50	32	22	10	0.298
<59.50	30	16	14	
Location				
Colon	24	2	22	0.468
Rectum	38	6	32	
Differentiation				
Well + Moderate	56	34	22	1.000
Poor	6	4	2	
Depth of invasion				
I	18	12	6	0.775
II	44	26	18	
Dukes' stage				
A+B+C	39	28	11	0.034[a]
D	23	10	13	
Lymph node metastasis				
Positive	34	20	14	0.795
Negative	28	18	10	
Liver metastasis				
Positive	24	11	13	0.028[a]
Negative	38	29	9	

I: Carcinoma *in situ* or tumors that invade the submucosa or the muscularis propria. II: Tumors that invade through the muscularis propria into the subserosa or deeper. [a]Statistically significant (P<0.05).

expression in the primary sites (34.23±13.05 vs. 57.47±4.34, P=0.0012). According to the Cox multivariate model which included patient gender, age, tumor location, differentiation, depth of invasion, Dukes' stage, lymph node metastasis, liver metastasis and PSMA7 protein expression, as shown in Table II, PSMA7 protein expression and liver metastasis were the only variables statistically significant for predicting survival (P=0.004 and 0.000, respectively).

Table II. PSMA7 protein expression shows an independent prognostic significance in Cox regression analysis.[a]

Variables[b]	Relative risk	Confidence interval (95%)	P-value
PSMA7 expression	5.057	1.682-15.201	0.004
Liver metastasis	8.790	3.026-25.533	0.000

[a]Method: Forward stepwise (Conditional LR). [b]Only the variables that had a statistically significant association with patient overall survival in multivariate analysis are shown. Factors included in the Cox regression analysis but without significant association are not shown.

Discussion

Proteasome-dependent proteolysis has emerged as a major regulatory mechanism that is broadly important during embryonic development, immune and stress responses, cell differentiation, cell cycle transitions, transcriptional regulation and apoptosis (6-8). The 26S proteasome has a molecular mass of ~2000 kDa and is formed from a 20S core complex which contains the protease catalytic activity, along with two polar 19S complexes which contain ATPase and non-ATPase regulators (9,10). The barrel-shaped 20S complex contains four rings, each of which is made up of seven different subunits. The two outer rings contain α-type subunits, whereas the inner rings contain ß-type subunits. The α-subunits lack any proteolytic activity, but they are required for proteasome assembly and 19S regulatory complex binding (11). They have been shown to function in substrate recognition and tethering of the substrate to the inner chamber of the 20S proteasome, where proteolysis takes place (12,13).

The deregulation of the proteasome pathway is often associated with cancer-related processes such as oncogenic transformation, tumor progression, escape from immune surveillance and drug resistance (14,15). The available information suggests that involvement in a malignant phenotype is conceivable for the proteasome subunit PSMA7 (16,17).

PSMA7 (also known as XAPC7, RC6-1 and HSPC in mammals) is one of the 7 proteasome α-subunits. Proteasomal activity was inhibited when PSMA7 associated with the X protein of the Hepatitis B virus (HBX), a substrate of proteasome (18). Subsequently, PSMA7 was shown to be important in the control of hepatitis C virus translation and downstream replication (16,19-21). The hypoxia-inducible factor-1α (HIF-1α) was identified as the first cellular target of PSMA7 and is an important transcription factor in the cellular response to oxygen tension. The HIF-1α physically interacts with PSMA7 and is targeted for proteasome-dependent degradation (22), suggesting an important regulatory mechanism in HIF-1α transactivation functions. Oxygen tension is an important factor for the regulation of mammalian genes that are involved in angiogenesis, vasculogenesis, glucose metabolism and apoptosis (23). Accumulating evidence indicates that the proteasome not only plays a proteolytic role in protein degradation but also a non-proteolytic role in transcription elongation, nuclear excision repair and protein trafficking (24-29). Lin *et al* (30) have reported that the overexpression of PSMA7 enhanced the androgen receptor (AR) transactivation in a dose-dependent manner and suggested that the proteasome system plays an important role in the regulation of AR activity in prostate cancer cells. Moreover, Ohnami *et al* (31) identified that PSMA7 was down-regulated in antisense K-ras-transduced pancreatic cancer cells with suppressed tumorigenicity. The mutations of the K-ras gene and the activation by point mutation is found at characteristically high frequencies of 70-90% in pancreatic cancer. The mutation incidence is 40-50% in colon cancer.

In this study, PSMA7 protein high expression was found to be significantly correlated with liver metastasis (P=0.028) by immunohistochemistry. Survival was significantly lower in patients with a PSMA7 protein high expression than in those with a PSMA7 protein low expression (P=0.0012). Moreover, in multivariate analysis, PSMA7 expression demonstrated an independent prognostic factor (P=0.004, relative risk 5.057; 95% confidence interval, 1.682-15.201). This is the first time that the relationship between PSMA7 expression and the clinicopathological features of colorectal cancer is reported. This relationship suggests that PSMA7 plays an important role in colorectal cancer progression. The evaluation of PSMA7 expression in primary colorectal cancer at the time of surgery may be a valuable tool for defining patients with a high risk of developing liver metastasis.

In addition, it has also been shown that p21WAF1/CIP1 is degraded by purified 20S proteasomes and that this is dependent on an interaction between the C terminus of p21WAF1/CIP1 and the C8 subunit of the 20S proteasome (32). PSMA7 and C8 are related α-subunits (33% identity) and have both been shown to function in the non-ubiquitin-dependent recognition and proteasome recruitment of several proteins with distinct consequences. We thererfore checked the p21WAF1/CIP1 protein expression in these cases, which, however, had no correlation with PSMA7 expression (data not shown).

We know that proteasome inhibitors induce cancer cell apoptosis, accompanied by the activation of several caspases, such as caspase-3 or -7 (33). However, apoptosis elicited by proteasome inhibitors is universal and not restricted only to cancer cells, so the molecular mechanisms by which PSMA7 plays a role in colorectal cancer progression will be investigated in detail in a future study which may provide a unique target site for the development of therapeutic drugs to inhibit active sites of proteasome subunits such as PSMA7 and prevent colorectal cancer metastasis.

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References

1. Gutman M and Fidler IJ: Biology of human colon cancer metastasis. World J Surg 19: 226-234, 1995.
2. Meijer GA, Hermsen MA, Baak JP, *et al*: Progression from colorectal adenoma to carcinoma is associated with non-random chromosomal gains as detected by comparative genomic hybridisation. J Clin Pathol 51: 901-909, 1998.
3. Ried T, Knutzen R, Steinbeck R, *et al*: Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. Genes Chromosomes Cancer 15: 234-245, 1996.
4. Schlegel J, Stumm G, Scherthan H, Bocker, T, Zirngibl H, Ruschoff J and Hofstadter F: Comparative genomic in situ hybridization of colon carcinomas with replication error. Cancer Res 55: 6002-6005, 1995.
5. Tsafrir D, Bacolod M, Selvanayagam Z, *et al*: Relationship of gene expression and chromosomal abnormalities in colorectal cancer. Cancer Res 66: 2129-2137, 2006.
6. Hershko A: Roles of ubiquitin-mediated proteolysis in cell cycle control. Curr Opin Cell Biol 9: 788-799, 1997.
7. Kloetzel PM: Generation of major histocompatibility complex class I antigens: functional interplay between proteasomes and TPPII. Nat Immunol 5: 661-669, 2004.
8. Varshavsky A: The N-end rule and regulation of apoptosis. Nat Cell Biol l5: 373-376, 2003.
9. Deveraux Q, Ustrell V, Pickart C and Rechsteiner M: A 26S protease subunit that binds ubiquitin conjugates. J Biol Chem 269: 7059-7061, 1994.
10. Hough R, Pratt G and Rechsteiner M: Purification of two high molecular weight proteases from rabbit reticulocyte lysate. J Biol Chem 262: 8303-8313, 1987.
11. Orlowski M and Wilk S: Catalytic activities of the 20S proteasome, a multicatalytic proteinase complex. Arch Biochem Biophys 383: 1-16, 2000.
12. Baumeister W, Walz J, Zuhl F and Seemuller E: The proteasome: paradigm of a self-compartmentalizing protease. Cell 92: 367-380, 1998.
13. Pickart CM and Cohen RE: Proteasomes and their kin: proteases in the machine age. Nat Rev Mol Cell Biol 5: 177-187, 2004.
14. Ciechanover A, Laszlo A, Bercovich B, Stancovski I, Alkalay I, Ben-Neriah Y and Orian A: The ubiquitin-mediated proteolytic system: involvement of molecular chaperones, degradation of oncoproteins, and activation of transcriptional regulators. Cold Spring Harb Symp Quant Biol 60: 491-501, 1995.
15. Spataro V, Norbury C and Harris AL: The ubiquitin-proteasome pathway in cancer. Br J Cancer 77: 448-455, 1998.
16. Huang J, Kwong J, Sun EC and Liang TJ: Proteasome complex as a potential cellular target of hepatitis B virus X protein. J Virol 70: 5582-5591, 1996.
17. Kumatori A, Tanaka K, Inamura N, *et al*: Abnormally high expression of proteasomes in human leukemic cells. Proc Natl Acad Sci USA 87: 7071-7075, 1990.
18. Hu Z, Zhang Z, Doo E, Coux O, Goldberg AL and Liang TJ: Hepatitis B virus X protein is both a substrate and a potential inhibitor of the proteasome complex. J Virol 73: 7231-7240, 1999.

19. Zhang Z, Torii N, Furusaka A, Malayaman N, Hu Z and Liang TJ: Structural and functional characterization of interaction between hepatitis B virus X protein and the proteasome complex. J Biol Chem 275: 15157-15165, 2000.
20. Ryo A, Suzuki Y, Arai M, *et al*: Identification and characterization of differentially expressed mRNAs in HIV type 1-infected human T cells. AIDS Res Hum Retroviruses 16: 995-1005, 2000.
21. Kruger M, Beger C, Welch PJ, Barber JR, Manns MP and Wong-Staal F: Involvement of proteasome alpha-subunit PSMA7 in hepatitis C virus internal ribosome entry site-mediated translation. Mol Cell Biol 21: 8357-8364, 2001.
22. Cho S, Choi YJ, Kim JM, Jeong ST, Kim JH, Kim SH and Ryu SE: Binding and regulation of HIF-1alpha by a subunit of the proteasome complex, PSMA7. FEBS Lett 498: 62-66, 2001.
23. Semenza GL: Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol 15: 551-578, 1999.
24. Pickart CM: Ubiquitin enters the new millennium. Mol Cell 8: 499-504, 2001.
25. Shenoy SK, McDonald PH, Kohout TA and Lefkowitz RJ: Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. Science 294: 1307-1313, 2001.
26. Yu A and Malek TR: The proteasome regulates receptor-mediated endocytosis of interleukin-2. J Biol Chem 276: 381-385, 2001.
27. Ferdous A, Gonzalez F, Sun L, Kodadek T and Johnston SA: The 19S regulatory particle of the proteasome is required for efficient transcription elongation by RNA polymerase II. Mol Cell 7: 981-991, 2001.
28. Gillette TG, Huang W, Russell SJ, Reed SH, Johnston SA and Friedberg EC: The 19S complex of the proteasome regulates nucleotide excision repair in yeast. Genes Dev 15: 1528-1539, 2001.
29. Lommel L, Chen L, Madura K and Sweder K: The 26S proteasome negatively regulates the level of overall genomic nucleotide excision repair. Nucleic Acids Res 28: 4839-4845, 2000.
30. Lin HK, Altuwaijri S, Lin WJ, Kan PY, Collins LL and Chang C: Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with coregulators in prostate cancer cells. J Biol Chem 277: 36570-36576, 2002.
31. Ohnami S, Matsumoto N, Nakano M, *et al*: Identification of genes showing differential expression in antisense K-ras-transduced pancreatic cancer cells with suppressed tumorigenicity. Cancer Res 59: 5565-5571, 1999.
32. Touitou R, Richardson J, Bose S, Nakanishi M, Rivett J and Allday MJ: A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8 alpha-subunit of the 20S proteasome. EMBO J 20: 2367-2375, 2001.
33. Hougardy BM, Maduro JH, van der Zee AG, de Groot DJ, van den Heuvel FA, de Vries EG and de Jong S: Proteasome inhibitor MG132 sensitizes HPV-positive human cervical cancer cells to rhTRAIL-induced apoptosis. Int J Cancer 118: 1892-1900, 2006.