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BRIEF ARTICLE

Early dynamic transcriptomic changes during preoperative radiotherapy in patients with rectal cancer: A feasibility study

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Abstract

AIM: To develop novel biomarkers of rectal radiotherapy, we measured gene expression profiles on biopsies taken before and during preoperative radiotherapy.

METHODS: Six patients presenting with a locally advanced rectal cancer (T>T2, N0/Nx, M0) eligible for preoperative radiotherapy (45 Gy in 25 fractions) were selected in a pilot study. Six tumor and 3 normal tissues biopsies were taken before and during radiotherapy, after a dose of 7.2 Gy at a median time of 1 h following irradiation (0:27-2:12). Tumor or normal tissue purity was assessed by a pathologist prior to RNA extraction. Mean RNA content was 23 μg/biopsy (14-37) before radiotherapy and 22.7 μg/biopsy (12-35) during radiotherapy. After RNA amplification, biopsies were analysed with 54K HG-U133A Plus 2.0 Affymetrix expression micro-arrays. Data were normalized according to MAS5 algorithm. A gene expression ratio was calculated as: (gene expression during radiotherapy - gene expression before radiotherapy)/gene expression before radiotherapy. Were selected genes that showed a ratio higher than ± 0.5 in all 6 patients.

RESULTS: Microarray analysis showed that preoperative radiotherapy significantly up-regulated 31 genes and down-regulated 6 genes. According to the Gene Ontology project classification, these genes are involved in protein metabolism (*ADAMDEC1*; *AKAP7*; *CAPN5*; *CLIC5*; *CPE*; *CREB3L1*; *NEDD4L*; *RAB27A*), ion transport (*AKAP7*; *ATP2A3*; *CCL28*; *CLIC5*; *F2RL2*; *NEDD4L*; *SLC6A8*), transcription (*AKAP7*; *CREB3L1*; *ISX*; *PABPC1L*; *TXNIP*), signal transduction (*CAPN5*; *F2RL2*; *RAB27A*; *TNFRSF11A*), cell adhesion (*ADAMDEC1*; *PXDN*; *SPON1*; *S100A2*), immune response (*CCL28*; *PXDN*; *TNFRSF11A*) and apoptosis (*ITM2C*; *PDCD4*; *PVT1*). Up-regulation of 3 genes (*CCL28*; *CLIC5*; *PDCD4*) was detected by 2 different probes and up-regulation of 2 genes (*RAB27A*; *TXNIP*) by 3 probes.

CONCLUSION: Micro-arrays can efficiently assess early transcriptomic changes during preoperative radiotherapy for rectal cancer, and may help better understand tumor radioresistance.

Core tip: To develop novel biomarkers of radiotherapy for rectal cancer, we measured gene expression profiles on biopsies taken before and during preoperative radiotherapy in a pilot study. Microarray analysis showed that preoperative radiotherapy significantly up-regulated 31 genes and down-regulated 6 genes, involved in protein metabolism, ion transport, transcription, signal transduction, cell adhesion, immune response and apoptosis. Micro-arrays could efficiently assess early transcriptomic changes during preoperative radiotherapy for rectal cancer. This may help better understand tumor radioresistance.

INTRODUCTION

In patients with rectal adenocarcinoma, preoperative radiotherapy (RT), either alone or combined with chemotherapy, reduces the 5-year rate of local recurrence by 5% to 10%[1]. However, owing to high inter-individual variation, 6-18 patients have to be treated in order to avoid one recurrence. Identifying patients likely to benefit is thus essential.

End outcomes after preoperative RT can hardly be predicted by analyzing the expression of known proteins on pre-treatment biopsies[2] or clinical parameters[3]. However, micro-array gene expression profiling can help define diagnostic, prognostic and predictive factors for response to RT[4]. Transcriptomic profiles obtained on pre-RT biopsies can be used to identify patients with rectal adenocarcinoma who are likely to relapse despite appropriate treatment[5]. Validation studies on larger cohorts are ongoing.

Sequential biopsies have highlighted changes in tumor dynamics (proliferation, cell cycle, apoptosis) during pelvic RT and identified treatment targets that might modify RT outcomes in patients with cervical cancer, where tumor access is easier than for the rectum[6,7]. Although sequential biopsies have also revealed histological changes in rectal mucosa due to radiation toxicity, data are few[8-11]. Very recently, biopsy specimens could be obtained 7 d after starting chemoradiotherapy and provided interesting biomarkers of response to treatment in rectal cancer patients[12].

A study of radiation-induced cellular and biochemical changes in rectal tumors might help better understand radiation-induced cell death and identify new targets for enhancing RT efficacy. We postulated that sequential biopsies could be used to detect transcriptional changes during preoperative RT of rectal cancer and help detect new predictors for response to radiation. A large-scale prospective study is needed to test this hypothesis. To eliminate the risk of increasing toxicity from repeated biopsy, we first conducted a pilot study to assess the safety of pre- and post-RT rectal tumor biopsies, and also the feasibility of detecting gene expression changes on biopsies from irradiated tumors.

MATERIALS AND METHODS

Patients

Patients presenting with locally advanced rectal cancer (T>T2, N0/Nx, M0) and eligible for preoperative RT were enrolled into the study. Exclusion criteria were: anticoagulant therapy, cardiac valvular disease, and pelvic pain from prior biopsies. All patients gave their informed written consent to the study, which was approved by the University of Nantes Institutional Review Board for human studies.

Biopsies

Patients were delivered 45 Gy (1.8 Gy/fraction, over 5 wk, 5 d per week). Six tumor and 3 normal tissue biopsies were taken from each patient before RT and one hour after a dose of 7.2 Gy (4th fraction) during RT. Patients were assessed for biopsy toxicity (infection or bleeding) during RT. Tumor purity was measured on tumor cell smears.

RNA isolation and microarray procedures

Tissues were frozen immediately in liquid nitrogen and disrupted using a mortar and pestle. Samples were homogenized in lysis buffer using a syringe and needle. Total RNA was prepared using the RNeasy® Mini kit (Qiagen, Valencia, CA, United States). The integrity of the RNA was assessed for each sample using an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, United States). Double-stranded complementary DNA (cDNA) and labeled complementary RNA were synthesized from the total RNA and hybridized to the Affymetrix Human U133 plus 2 gene chips (Affymetrix, Santa Clara, CA, United States). The chips were further processed and scanned according to the manufacturer's protocol. The arrays were scanned with a laser scanner and the data was visualized and normalized using the MAS 5.0 Affymetrix software (Affymetrix, Santa Clara, CA, United States). Over- and under-expressed genes were classified by Gene Ontology category[13].

Statistical analysis

Data were normalized according to the MAS5 algorithm. The ratio "gene expression during RT/gene expression before RT" was calculated. Genes with a ratio > 2.5 or < 0.4 in all patients and a false discovery rate (FDR) of < 11%, as estimated by significance analysis of microarrays, were selected.

Table 1 Patient characteristics

Patient	Age (yr)	Disease stage	> G1 radiotherapy toxicity	Surgical toxicity	Surgical stage	Outcome at 2 yr of follow-up	Tumor purity in biopsies	Mean RNA content (μg)	
								Pre-RT biopsies	Early-RT biopsies
1	66	uT2Nx	No	No	ypT2N0	NED	100%	18.0	19.7
2	55	uT3N1	No	No	ypT4N1	NED	100%	27.0	12.6
3	84	uT3N1	No	No	ypT3N1	M1	100%	21.0	35.8
4	78	uT3N1	No	No	ypT2N0	NED	100%	37.1	28.7
5	60	uT3N0	No	No	ypT3N1	NED	100%	20.0	19.4
6	67	uT3N0	No	No	ypT2N1	NED	100%	14.2	19.9

NED: Non evolutive disease, M1: Metastatic disease; RT: Radiotherapy.

Table 2 Up- or down-regulated genes in rectal cancer biopsies

Fold	False discovery rate	ID	Gene title	Symbol
Up-regulated genes				
2.649	0	AI827789	Anterior gradient homolog 3 (Xenopus)	AGR3
3.427	0	AA743462	-	-
2.524	0	NM_006472	Thioredoxin interacting protein	TXNIP
5.514	0	AB018305	Spondin 1, extracellular matrix protein	SPON1
2.95	7.551	NM_152315	Family with sequence similarity 55, A	FAM55A
3.289	7.551	AL536553	Neural precursor cell expressed, developmentally down-regulated 4-like	NEDD4L
2.684	7.551	AF055009	cAMP responsive element binding protein 3-like 1	CREB3L1
3.111	7.551	AL137063	A kinase (PRKA) anchor protein 7	AKAP7
2.717	7.551	AF266504	Chemokine (C-C motif) ligand 28	CCL28
2.546	8.39	BF589413	FERM domain containing 3	FRMD3
2.637	8.39	AA554045	Polypeptide N-acetylgalactosaminyltransferase 12	GALNT12
3.047	8.39	NM_030926	Integral membrane protein 2C	ITM2C
2.775	8.39	AW026379	Tumor necrosis factor receptor superfamily, member 11a	TNFRSF11A
2.609	8.39	U38654	RAB27A, member RAS family	RAB27A
2.629	8.39	NM_003570	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase pseudogene	CMAH
2.521	8.58	AV728268	Chromosome 11 open reading frame 32	C11orf32
2.579	8.58	NM_016929	Chloride intracellular channel 5	CLIC5
2.541	10.487	NM_016548	Golgi membrane protein 1	GOLM1
2.728	10.487	AW162846	Calcium/calmodulin-dependent protein kinase Ⅱ inhibitor 1	CAMK2N1
2.806	10.487	AI659927	prostate androgen-regulated mucin-like protein 1	PARM1
2.896	10.487	NM_024709	Chromosome 1 open reading frame 115	C1orf115
3.333	10.487	NM_005629	Solute carrier family 6 member 8	SLC6A8
2.869	10.487	NM_019062	Ring finger protein 186	RNF186
3.174	10.487	AK025181	Intestine-specific homeobox	ISX
3.968	10.487	AB007899	Neural precursor cell expressed, developmentally down-regulated 4-like	NEDD4L
8.2	10.487	NM_001873	Carboxypeptidase E	CPE
4.845	10.487	NM_014479	ADAM-like, decysin 1	ADAMDEC1
2.56	10.487	BF195709	Calpain 5	CAPN5
2.601	10.487	NM_014456	Programmed cell death 4	PDCD4
2.931	10.787	AW971415	cDNA clone IMAGE:5745639	-
2.787	10.787	NM_005173	ATPase, Ca++ transporting, ubiquitous	ATP2A3
Down-regulated genes				
0.265	8.58	AI378647	Coagulation factor Ⅱ (thrombin) receptor-like 2	F2RL2
0.356	8.58	BG200951	Pvt1 oncogene homolog, MYC activator	PVT1
0.322	10.787	AL109839	Poly(A) binding protein, cytoplasmic 1-like	PABPC1L
0.356	10.787	NM_005978	S100 calcium binding protein A2	S100A2
0.373	10.787	AL041761	Transcribed locus	-
0.38	10.787	D86983	Peroxidasin homolog (Drosophila)	PXDN

RESULTS

Seven patients with rectal cancer (median age: 66 years, range 55-84 years) were included in the study but one later refused to participate (Table 1). Six patients underwent biopsy before and during RT. The median time of the biopsy was 1 h after the 4th RT session (0:27-2:12). No grade 2 biopsy-induced toxicity was reported. Surgery was performed at a median time of 6 wk after RT. No grade 2 intra-operative toxicity was reported.

Mean RNA content was 23 μg/biopsy (range 14-37 μg/biopsy) before RT and 22.7 μg/biopsy (range 12-35 μg/biopsy) during RT (Table 1). Microarray analysis showed that preoperative RT significantly up-regulated 31 genes and down-regulated 6 genes (the full names of the genes are given in Table 2). According to the Gene Ontology project classification, these genes are involved in protein metabolism (*ADAMDEC1*; *AKAP7*; *CAPN5*;

CLIC5; *CPE*; *CREB3L1*; *NEDD4L*; *RAB27A*), ion transport (*AKAP7*; *ATP2A3*; *CCL28*; *CLIC5*; *F2RL2*; *NEDD4L*; *SLC6A8*), transcription (*AKAP7*; *CREB3L1*; *ISX*; *PABPC1L*; *TXNIP*), signal transduction (*CAPN5*; *F2RL2*; *RAB27A*; *TNFRSF11A*), cell adhesion (*AD-AMDEC1*; *PXDN*; *SPON1*; *S100A2*), immune response (*CCL28*; *PXDN*; *TNFRSF11A*) and apoptosis (*ITM2C*; *PDCD4*; *PVT1*)[13]. Up-regulation of 3 genes (*CCL28*; *CLIC5*; *PDCD4*) was detected by 2 different probes and up-regulation of 2 genes (*RAB27A*; *TXNIP*) by 3 probes.

DISCUSSION

Biopsies taken during preoperative RT for rectal cancer were not associated with enhanced toxicity (infection or bleeding). cDNA micro-array analysis on tumor biopsies uncontaminated by normal tissue was possible provided that the extracted RNA was amplified.

Analysis of gene transcription pre- and post-RT detected many up-regulated genes involved in tumor development such as *GOLM1* (prostate cancer)[14], *CAMK2N1* (a tumor suppressor gene in colon cancer)[15], *AGR3* (breast cancer)[16] and *PDCD4* (lung and ovarian cancer)[17]. However and contrarily to *in vitro* studies[18], it did not detect genes thought to be involved in cell repair after radiation-induced damage.

On the other hand, we detected several early response genes mostly involved in stress such as *CPE* which protects against oxidative stress-induced cell death and *ROS*-induced cell apoptosis[19], hypoxia-induced *TXNIP* which is regulated by hypoxia-induction factor 1[20,21], *CREB3L1* which codes for a protein that is cleaved in response to stress on the endoplasmic reticulum[22], and *ITM2C* which is over-expressed after alpha radiation but whose role is not known[23].

Many genes implicated in ion channel regulation were up-regulated during radiotherapy, such as *NEDD4L* which regulates sodium channels *via* the Wnt/beta-catenin signaling pathway during colon carcinogenesis[24], *SLC6A8* which codes for a Na^+Cl^- dependent creatine transporter[25], *ATP2A3* which encodes an intracellular pump participating in Ca^{2+} sequestration, and *CLIC5*, a member of the chloride intracellular channel gene family, structurally homologous to the glutathione-S-transferase superfamily[26].

Among up-regulated genes, we were surprised to find many that are implicated in the immune response, such as *ADAMDEC1* (decysin) which plays a key role in the interaction between dendritic cells and germinal center T-helper cells[27], *ITM2C* which is involved in TNF-induced cell death[28], *TNFRSF11A* which codes for a protein of the TNF receptor superfamily, *CCL28*, a member of the small cytokine CC gene subfamily[29], *RAB27* which regulates exocytosis of neutrophil granules[30], and *PDCD4* (programmed cell death 4) which is regulated by several interleukins (IL-2, IL-15 and IL-12) in natural killer and T cells[31]. This was the only gene of our list that has been reported to be a predictor of response to preoperative radiochemotherapy in pre-treatment biopsies of patients with rectal cancer[32]. It codes for a tumor suppressor protein that inhibits translation initiation factor eIF4A which lies downstream of the AKT/mTOR pathway and plays a role in response to DNA damage[33-35]. *PDCD4* mRNA levels during RT may thus be an interesting surrogate marker in studies of mTOR inhibitors plus RT for rectal cancer.

Three of the up-regulated genes we detected (*GALNT12*, *CMAH* and *SPON1*) are involved in metabolic processes and interactions with glycans, and would seem to occupy a key role in triggering an immune response[36]. SPON1 protein belongs the thrombospondin type 1 repeat superfamily of proteins that bind transforming growth factor-β[37]. By analogy with *SPON2*, it might be involved in mechanisms of activation of innate and adaptive immune responses[38].

Apart from *PDCD4* and *SPON1*, we identified at least two other genes that might constitute novel therapeutic targets in preoperative RT for rectal cancer. *TXNIP* (the gene coding for thioredoxin interacting protein) is a key regulator of redox status. Thioredoxin is released from cells in response to oxidative stress and its plasma or serum level is a good marker of cancer-related oxidative stress. Because thioredoxin mediates redox-induced cell death in colon cancer cells, it is an attractive target for anti-tumor therapy[39]. Thioredoxin inhibitors are currently under investigation[40]. *Rab27A* gene expression was also up-regulated during RT. Rab27A is a small G protein which regulates secretory activity in colon cancer cells and promotes invasiveness and metastasis in breast cancer cells[41,42]. Novel inhibitors of Rab geranylgeranyl-transferase might thus prove to be inhibitors of rectal tumor cell proliferation and RT enhancers[43]. Interestingly, the highly up-regulated clone 5745639 was found by Blast analysis 2022 bp from the 3' end of the Ras-related protein Rab-27A.

A limitation of our study is the small number of tumors analyzed. Our results are thus essentially hypothesis generating. Future attention should focus on the genes that have yielded the most robust results (low FDR and detected by several probes).

In conclusion, biopsies taken during early RT sessions may be used for *in vivo* measurement of tumor sensitivity and have low morbidity. Many genes involved in triggering immune response seem to be expressed during RT. We hypothesize that the changes in gene profiles observed early during RT may help predict rectal tumor response to preoperative RT, whether alone or combined with chemotherapy. Gene profiling may help: (1) identify predictors of resistance to RT that will enable exclusion of patients likely to be cured by surgery alone; (2) assess the validity of surrogate markers during phase I testing of new radiosensitizing drugs which are used together with RT to treat selected resistant rectal tumors; and (3) define new targets for improving the efficacy of preoperative RT of rectal cancer. To test our hypothesis, larger cohorts of patients are needed and the best time for gene

profiling during and after RT needs to be determined.

COMMENTS

Background
Because 6-18 patients have to be treated in order to avoid one recurrence, identifying patients with rectal adenocarcinoma likely to benefit from preoperative radiotherapy is essential. End outcomes after preoperative radiotherapy (RT) can hardly be predicted by analyzing pre-treatment clinical parameter, the expression of known proteins or transcriptomic profiles.

Research frontiers
Sequential biopsies have highlighted changes in tumor dynamics during pelvic RT and identified treatment targets that might modify RT outcomes. The authors postulated that sequential biopsies could be used to detect transcriptional changes during preoperative RT of rectal cancer and help detect new predictors for response to radiation.

Innovations and breakthroughs
Analysis of gene transcription pre- and post-RT detected many up-regulated genes involved in tumor development. However and contrarily to *in vitro* studies, it did not detect genes involved in DNA repair. Several early response genes mostly involved in stress response and genes involved in ion channel regulation were up-regulated during radiotherapy. Surprisingly, many genes that are implicated in the immune response were found to be up-regulated.

Applications
Biopsies taken during early RT sessions can used for *in vivo* measurement of tumor sensitivity and have low morbidity. Many genes involved in triggering immune response seem to be expressed during RT. The changes in gene profiles observed early during RT may help predict rectal tumor response to preoperative RT, whether alone or combined with chemotherapy.

Terminology
DNA microarray is a collection of microscopic DNA spots attached to a solid surface used to measure the expression levels of large numbers of genes in a tumor sample.

Peer review
In this study, the authors examined the transcriptomic changes during preoperative radiotherapy in French patients with rectal cancer. In general, this is a good attempt to seek transcriptomic biomarkers for patients with rectal cancer and can provide important information for clinicians.

REFERENCES

1   **Supiot S**, Bennouna J, Rio E, Meurette G, Bardet E, Buecher B, Dravet F, Le Neel JC, Douillard JY, Mahé MA, Lehur PA. Negative influence of delayed surgery on survival after preoperative radiotherapy in rectal cancer. *Colorectal Dis* 2006; **8**: 430-435 [PMID: 16684088 DOI: 10.1111/j.1463-1318.2006.00990.x]

2   **Smith FM**, Reynolds JV, Miller N, Stephens RB, Kennedy MJ. Pathological and molecular predictors of the response of rectal cancer to neoadjuvant radiochemotherapy. *Eur J Surg Oncol* 2006; **32**: 55-64 [PMID: 16324817 DOI: 10.1016/j.ejso.2005.09.010]

3   **Park CH**, Kim HC, Cho YB, Yun SH, Lee WY, Park YS, Choi DH, Chun HK. Predicting tumor response after preoperative chemoradiation using clinical parameters in rectal cancer. *World J Gastroenterol* 2011; **17**: 5310-5316 [PMID: 22219601 DOI: 10.3748/wjg.v17.i48.5310]

4   **Ogawa K**, Murayama S, Mori M. Predicting the tumor response to radiotherapy using microarray analysis (Review). *Oncol Rep* 2007; **18**: 1243-1248 [PMID: 17914580]

5   **Akiyoshi T**, Kobunai T, Watanabe T. Predicting the response to preoperative radiation or chemoradiation by a microarray analysis of the gene expression profiles in rectal cancer. *Surg Today* 2012; **42**: 713-719 [PMID: 22706722 DOI: 10.1007/s00595-012-0223-8]

6   **Durand RE**, Aquino-Parsons C. Predicting response to treatment in human cancers of the uterine cervix: sequential biopsies during external beam radiotherapy. *Int J Radiat Oncol Biol Phys* 2004; **58**: 555-560 [PMID: 14751527]

7   **Ishikawa H**, Ohno T, Kato S, Wakatsuki M, Iwakawa M, Ohta T, Imai T, Mitsuhashi N, Noda SE, Nakano T, Tsujii H. Cyclooxygenase-2 impairs treatment effects of radiotherapy for cervical cancer by inhibition of radiation-induced apoptosis. *Int J Radiat Oncol Biol Phys* 2006; **66**: 1347-1355 [PMID: 16979845 DOI: 10.1016/j.ijrobp.2006.07.007]

8   **Hovdenak N**, Fajardo LF, Hauer-Jensen M. Acute radiation proctitis: a sequential clinicopathologic study during pelvic radiotherapy. *Int J Radiat Oncol Biol Phys* 2000; **48**: 1111-1117 [PMID: 11072170 DOI: 10.1016/S0360-3016(00)00744-6]

9   **Sedgwick DM**, Howard GC, Ferguson A. Pathogenesis of acute radiation injury to the rectum. A prospective study in patients. *Int J Colorectal Dis* 1994; **9**: 23-30 [PMID: 8027619]

10 **Flam M**, John M, Pajak TF, Petrelli N, Myerson R, Doggett S, Quivey J, Rotman M, Kerman H, Coia L, Murray K. Role of mitomycin in combination with fluorouracil and radiotherapy, and of salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. *J Clin Oncol* 1996; **14**: 2527-2539 [PMID: 8823332]

11 **Hovdenak N**, Wang J, Sung CC, Kelly T, Fajardo LF, Hauer-Jensen M. Clinical significance of increased gelatinolytic activity in the rectal mucosa during external beam radiation therapy of prostate cancer. *Int J Radiat Oncol Biol Phys* 2002; **53**: 919-927 [PMID: 12095558 DOI: 10.1016/S0360-3016(02)02808-0]

12 **Suzuki T**, Sadahiro S, Tanaka A, Okada K, Kamata H, Kamijo A, Murayama C, Akiba T, Kawada S. Biopsy specimens obtained 7 days after starting chemoradiotherapy (CRT) provide reliable predictors of response to CRT for rectal cancer. *Int J Radiat Oncol Biol Phys* 2013; **85**: 1232-1238 [PMID: 23158058 DOI: 10.1016/j.ijrobp.2012.09.031]

13 **Ashburner M**, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; **25**: 25-29 [PMID: 10802651 DOI: 10.1038/75556]

14 **Varambally S**, Laxman B, Mehra R, Cao Q, Dhanasekaran SM, Tomlins SA, Granger J, Vellaichamy A, Sreekumar A, Yu J, Gu W, Shen R, Ghosh D, Wright LM, Kladney RD, Kuefer R, Rubin MA, Fimmel CJ, Chinnaiyan AM. Golgi protein GOLM1 is a tissue and urine biomarker of prostate cancer. *Neoplasia* 2008; **10**: 1285-1294 [PMID: 18953438 DOI: 10.1593/neo.08922]

15 **Wang C**, Li N, Liu X, Zheng Y, Cao X. A novel endogenous human CaMKII inhibitory protein suppresses tumor growth by inducing cell cycle arrest via p27 stabilization. *J Biol Chem* 2008; **283**: 11565-11574 [PMID: 18305109 DOI: 10.1074/jbc.M800436200]

16 **Fletcher GC**, Patel S, Tyson K, Adam PJ, Schenker M, Loader JA, Daviet L, Legrain P, Parekh R, Harris AL, Terrett JA. hAG-2 and hAG-3, human homologues of genes involved in differentiation, are associated with oestrogen receptor-positive breast tumours and interact with metastasis gene C4.4a and dystroglycan. *Br J Cancer* 2003; **88**: 579-585 [PMID: 12592373 DOI: 10.1038/sj.bjc.6600740]

17 **Wang X**, Wei Z, Gao F, Zhang X, Zhou C, Zhu F, Wang Q, Gao Q, Ma C, Sun W, Kong B, Zhang L. Expression and prognostic significance of PDCD4 in human epithelial ovarian carcinoma. *Anticancer Res* 2008; **28**: 2991-2996 [PMID: 19031945]

18 **Kumaraswamy S**, Chinnaiyan P, Shankavaram UT, Lü X, Camphausen K, Tofilon PJ. Radiation-induced gene translation profiles reveal tumor type and cancer-specific components. *Cancer Res* 2008; **68**: 3819-3826 [PMID: 18483266 DOI: 10.1158/0008-5472.CAN-08-0016]

19   **Koshimizu H**, Senatorov V, Loh YP, Gozes I. Neuroprotective protein and carboxypeptidase E. *J Mol Neurosci* 2009; **39**: 1-8 [PMID: 19165633 DOI: 10.1007/s12031-008-9164-5]

20   **Baker AF**, Koh MY, Williams RR, James B, Wang H, Tate WR, Gallegos A, Von Hoff DD, Han H, Powis G. Identification of thioredoxin-interacting protein 1 as a hypoxia-inducible factor 1alpha-induced gene in pancreatic cancer. *Pancreas* 2008; **36**: 178-186 [PMID: 18376310 DOI: 10.1097/MPA.0b013e31815929fe]

21   **Li X**, Rong Y, Zhang M, Wang XL, LeMaire SA, Coselli JS, Zhang Y, Shen YH. Up-regulation of thioredoxin interacting protein (Txnip) by p38 MAPK and FOXO1 contributes to the impaired thioredoxin activity and increased ROS in glucose-treated endothelial cells. *Biochem Biophys Res Commun* 2009; **381**: 660-665 [PMID: 19254690 DOI: 10.1016/j.bbrc.2009.02.132]

22   **Saito A**, Hino S, Murakami T, Kondo S, Imaizumi K. A novel ER stress transducer, OASIS, expressed in astrocytes. *Antioxid Redox Signal* 2007; **9**: 563-571 [PMID: 17330990 DOI: 10.1089/ars.2006.1520]

23   **Seidl C**, Port M, Apostolidis C, Bruchertseifer F, Schwaiger M, Senekowitsch-Schmidtke R, Abend M. Differential gene expression triggered by highly cytotoxic alpha-emitter-immunoconjugates in gastric cancer cells. *Invest New Drugs* 2010; **28**: 49-60 [PMID: 19139817 DOI: 10.1007/s10637-008-9214-4]

24   **Lee HS**, Park MH, Yang SJ, Park KC, Kim NS, Kim YS, Kim DI, Yoo HS, Choi EJ, Yeom YI. Novel candidate targets of Wnt/beta-catenin signaling in hepatoma cells. *Life Sci* 2007; **80**: 690-698 [PMID: 17157329 DOI: 10.1016/j.lfs.2006.10.024]

25   **Ireland Z**, Russell AP, Wallimann T, Walker DW, Snow R. Developmental changes in the expression of creatine synthesizing enzymes and creatine transporter in a precocial rodent, the spiny mouse. *BMC Dev Biol* 2009; **9**: 39 [PMID: 19570237 DOI: 10.1186/1471-213X-9-39]

26   **Cromer BA**, Morton CJ, Board PG, Parker MW. From glutathione transferase to pore in a CLIC. *Eur Biophys J* 2002; **31**: 356-364 [PMID: 12202911 DOI: 10.1007/s00249-002-0219-1]

27   **Fritsche J**, Müller A, Hausmann M, Rogler G, Andreesen R, Kreutz M. Inverse regulation of the ADAM-family members, decysin and MADDAM/ADAM19 during monocyte differentiation. *Immunology* 2003; **110**: 450-457 [PMID: 14632642 DOI: 10.1046/j.1365-2567.2003.01754.x]

28   **Wu H**, Liu G, Li C, Zhao S. bri3, a novel gene, participates in tumor necrosis factor-alpha-induced cell death. *Biochem Biophys Res Commun* 2003; **311**: 518-524 [PMID: 14592447 DOI: 10.1016/j.bbr.2011.03.031]

29   **Williams IR**. Chemokine receptors and leukocyte trafficking in the mucosal immune system. *Immunol Res* 2004; **29**: 283-292 [PMID: 15181289]

30   **Herrero-Turrión MJ**, Calafat J, Janssen H, Fukuda M, Mollinedo F. Rab27a regulates exocytosis of tertiary and specific granules in human neutrophils. *J Immunol* 2008; **181**: 3793-3803 [PMID: 18768832]

31   **Azzoni L**, Zatsepina O, Abebe B, Bennett IM, Kanakaraj P, Perussia B. Differential transcriptional regulation of CD161 and a novel gene, 197/15a, by IL-2, IL-15, and IL-12 in NK and T cells. *J Immunol* 1998; **161**: 3493-3500 [PMID: 9759869]

32   **Kim IJ**, Lim SB, Kang HC, Chang HJ, Ahn SA, Park HW, Jang SG, Park JH, Kim DY, Jung KH, Choi HS, Jeong SY, Sohn DK, Kim DW, Park JG. Microarray gene expression profiling for predicting complete response to preoperative chemoradiotherapy in patients with advanced rectal cancer. *Dis Colon Rectum* 2007; **50**: 1342-1353 [PMID: 17665260 DOI: 10.1007/s10350-007-277-7]

33   **Dorrello NV**, Peschiaroli A, Guardavaccaro D, Colburn NH, Sherman NE, Pagano M. S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. *Science* 2006; **314**: 467-471 [PMID: 17053147 DOI: 10.1126/science.1130276]

34   **Woodard J**, Sassano A, Hay N, Platanias LC. Statin-dependent suppression of the Akt/mammalian target of rapamycin signaling cascade and programmed cell death 4 up-regulation in renal cell carcinoma. *Clin Cancer Res* 2008; **14**: 4640-4649 [PMID: 18628479 DOI: 10.1158/1078-0432.CCR-07-5232]

35   **Bitomsky N**, Wethkamp N, Marikkannu R, Klempnauer KH. siRNA-mediated knockdown of Pdcd4 expression causes upregulation of p21(Waf1/Cip1) expression. *Oncogene* 2008; **27**: 4820-4829 [PMID: 18427550 DOI: 10.1038/onc.2008.115]

36   **Rubartelli A**, Lotze MT. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol* 2007; **28**: 429-436 [PMID: 17845865 DOI: 10.1016/j.it.2007.08.004]

37   **Schultz-Cherry S**, Lawler J, Murphy-Ullrich JE. The type 1 repeats of thrombospondin 1 activate latent transforming growth factor-beta. *J Biol Chem* 1994; **269**: 26783-26788 [PMID: 7929414]

38   **Li Y**, Cao C, Jia W, Yu L, Mo M, Wang Q, Huang Y, Lim JM, Ishihara M, Wells L, Azadi P, Robinson H, He YW, Zhang L, Mariuzza RA. Structure of the F-spondin domain of mindin, an integrin ligand and pattern recognition molecule. *EMBO J* 2009; **28**: 286-297 [PMID: 19153605 DOI: 10.1038/emboj.2008.288]

39   **Sun Y**, Rigas B. The thioredoxin system mediates redox-induced cell death in human colon cancer cells: implications for the mechanism of action of anticancer agents. *Cancer Res* 2008; **68**: 8269-8277 [PMID: 18922898 DOI: 10.1158/0008-5472.CAN-08-2010]

40   **Bradshaw TD**, Matthews CS, Cookson J, Chew EH, Shah M, Bailey K, Monks A, Harris E, Westwell AD, Wells G, Laughton CA, Stevens MF. Elucidation of thioredoxin as a molecular target for antitumor quinols. *Cancer Res* 2005; **65**: 3911-3919 [PMID: 15867391 DOI: 10.1158/0008-5472.CAN-04-4141]

41   **Saxena S**, Singh M, Engisch K, Fukuda M, Kaur S. Rab proteins regulate epithelial sodium channel activity in colonic epithelial HT-29 cells. *Biochem Biophys Res Commun* 2005; **337**: 1219-1223 [PMID: 16236259]

42   **Wang JS**, Wang FB, Zhang QG, Shen ZZ, Shao ZM. Enhanced expression of Rab27A gene by breast cancer cells promoting invasiveness and the metastasis potential by secretion of insulin-like growth factor-II. *Mol Cancer Res* 2008; **6**: 372-382 [PMID: 18337447 DOI: 10.1158/1541-7786.MCR-07-0162]

43   **Watanabe M**, Fiji HD, Guo L, Chan L, Kinderman SS, Slamon DJ, Kwon O, Tamanoi F. Inhibitors of protein geranylgeranyltransferase I and Rab geranylgeranyltransferase identified from a library of allenoate-derived compounds. *J Biol Chem* 2008; **283**: 9571-9579 [PMID: 18230616 DOI: 10.1074/jbc.M706229200]