

Integrative genomic analyses of the histamine H1 receptor and its role in cancer prediction

MINGHAI WANG^{1*}, XIAOLONG WEI^{2*}, LIANGHUI SHI¹, BIN CHEN¹, GUOHAI ZHAO¹ and HAIWEI YANG³

¹Department of General Surgery, The First Affiliated Yijishan Hospital of Wannan Medical College, Wuhu 241001;

²Department of Pathology, Cancer Hospital of Shantou University Medical College, Shantou 515041;

³Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, P.R. China

Received October 15, 2013; Accepted January 23, 2014

DOI: 10.3892/ijmm.2014.1649

Abstract. The human histamine receptor H1 (HRH1) gene is located on chromosome 3p25 and encodes for a 487 amino acid G protein-coupled receptor (GPCR) with a long third intracellular loop (IL3). The HRH1 predominantly couples to $G\alpha_{q/11}$ proteins, leading to the activation of phospholipase C (PLC) and subsequent release of the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) followed by the activation of PKC and the release of $[Ca^{2+}]_i$. In the present study, we identified HRH1 genes from 14 vertebrate genomes and found that HRH1 exists in all types of vertebrates including fish, amphibians, birds and mammals. We identified 88 SNPs including 4 available alleles disrupting an existing exonic splicing enhancer and 84 SNPs causing missense mutation, which may impact the effect of histamine on the HRH1 protein. We found that the human HRH1 gene was expressed in many tissues or organs, and predominant expression of HRH1 was shown in the bone marrow, whole blood, lymph node, thymus, brain, cerebellum, retina, spinal cord, heart, smooth muscle, skeletal muscle, small intestine, colon, adipocytes, kidney, liver, lung, pancreas, thyroid salivary gland, skin, ovary, uterus, placenta, prostate and testis. When searched in the PrognosScan database, human HRH1 was also found to be expressed in bladder cancer, blood cancer, brain cancer, breast cancer, colorectal cancer, eye cancer, head and neck cancer, lung cancer, ovarian cancer, skin cancer and soft tissue cancer tissues. The relationship

between the expression of HRH1 and prognosis was found to vary in different types of cancers, even in the same cancer from different databases. This implies that the function of HRH1 in these tumors may be multidimensional. GR, STAT5A and c-Myb regulatory transcription factor binding sites were identified in the HRH1 gene upstream (promoter) region, which may be involved in the effect of HRH1 in tumors.

Introduction

Histamine is a ubiquitous messenger molecule released from mast cells, enterochromaffin-like cells and neurons. Its various actions are mediated by histamine receptors H1, H2, H3 and H4 (1). The bovine histamine H1 receptor (HRH1) was the first HRH1 gene to be cloned, soon to be followed by other species, including human HRH1. The human HRH1 gene is located on chromosome 3p25 and encodes for a 487 amino acid G protein-coupled receptor (GPCR) with a long third intracellular loop (IL3) (1). The HRH1 predominantly couples to $G\alpha_{q/11}$ proteins, leading to the activation of phospholipase C (PLC) and subsequent release of the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) followed by the activation of protein kinase C (PKC) and the release of $[Ca^{2+}]_i$ (1). HRH1 is expressed in a wide variety of tissues including the gastrointestinal tract, the central nervous system, the immunological system, and the cardiovascular and genitourinary systems (2-5).

HRH1 is involved in a wide array of processes including thermal regulation, memory and learning, and control of the sleep-wake cycle, food intake, and emotional and aggressive behaviors. HRH1 is also involved in the pathological process of allergy, including allergic rhinitis, atopic dermatitis, anaphylaxis and asthma, and also has a role in autoimmune diseases and malignancy (2-7). Histamine is involved in cell proliferation, and key events in tumor development and progression have been extensively investigated (8,9). It has been found that HIR stimulates proliferation of breast carcinoma, melanoma and astrocytoma tumor cells (10-12). In this context, the investigation of HIR and its antagonists support a clear rationale for future supportive anticancer therapies. However, a comprehensive investigation of whether HRH1 is involved in the formation of various tumors has not yet been carried out.

Correspondence to: Dr Haiwei Yang, Department of Urology, The First Affiliated Hospital of Nanjing Medical University, No. 300 Guangzhou Road, Nanjing 210029, P.R. China
E-mail: hwyang2002@163.com

Professor Guohai Zhao, Department of General Surgery, The First Affiliated Yijishan Hospital of Wannan Medical College, 2 West Zheshan Road, Wuhu 241001, P.R. China
E-mail: wangmh0410@sina.com

*Contributed equally

Key words: HRH1, comparative genomics, cancer, prognosis, meta-analysis

In the present study, we identified HRH1 genes from the human, chimpanzee, macaque, orangutan, dog, cow, horse, mouse, rat, opossum, chicken, *Xenopus tropicalis*, zebrafish and fugu by comparative genomic analyses. A search for conserved transcription factor-binding sites within the promoter regions of the human HRH1 gene was then carried out. The expression data, functional relevant single nucleotide polymorphisms (SNPs) and comparative proteomic analyses were conducted. Furthermore, meta-analysis of the prognostic value of HRH1 genes in various types of cancers was also performed.

Materials and methods

Identification of novel HRH1 genes in vertebrate genomes and integrative genomic analyses. A search for HRH1 genes was carried out in the genome sequences of the human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), macaque (*Macaca mulatta*), orangutan (*Pongo pygmaeus*), dog (*Canis familiaris*), cow (*Bos taurus*), horse (*Equus caballus*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), opossum (*Monodelphis domestica*), chicken (*Gallus gallus*), *Xenopus tropicalis*, zebrafish (*Danio rerio*) and fugu (*Takifugu rubripes*) by the method described before using the human HRH1 gene (CAA84380) as a query. The assemblies used were human NCBI36, chimpanzee CHIMP2.1, macaque MMUL 1.0, orangutan PPYG2, dog CanFam 2.0, cow Btau_4.0, horse Equ Cab 2, mouse NCBI m37, rat RGSC 3.4, opossum monDom5, chicken WASHUC2, *X. tropicalis* JGI 4.1, zebrafish Zv8 and fugu FUGU 4.0. The identified putative HRH1 genes were BLASTed against the nr database of GenBank to confirm that the best hits were HRH1 genes (13-15). Conserved transcription factor-binding sites within the promoter region of the human HRH1 gene were obtained from SABiosciences' proprietary database which combines Text Mining Application and data from the UCSC Genome Browser.

Comparative proteomic analyses of HRH1 proteins. The amino acid sequences of HRH1 were deduced from the identified HRH1 genes and aligned using Clustal X 1.8 software (16). The phylogenetic tree of HRH1 was obtained by using ML (maximum likelihood) (PHYML v2.4.4) (17) and NJ (neighbor-joining) (MEGA 3.0) (18) methods, and the reliability of the tree was evaluated by the bootstrap method with 1,000 replications. The program Codeml implemented in the PAML 3.14 b software package was used to investigate whether HRH1 proteins are under positive selection (19). Six models of codon substitution, M0 (one-ratio), M1a (NearlyNeutral), M2a (PositiveSelection), M3 (discrete), M7 (β), and M8 (β and ω) were used in the analysis (20).

Functional relevant SNP evaluation of the human HRH1 gene. Functional relevant SNPs (single nucleotide polymorphisms) of the human HRH1 gene were identified as previously described (13-15,21). The SNPs were extracted from Ensembl (<http://www.ensembl.org>) and NCBI's SNPdb (<http://www.ncbi.nlm.nih.gov>). The SNPs that could disrupt ESE/ESS (exonic splicing enhancer/exonic splicing silencer) motifs and cause missense mutation were also identified.

In silico expression analyses of the human HRH1 gene. Expressed sequence tags (ESTs) derived from the human HRH1 gene were searched for using the BLAST programs as previously described (22-25). Human HRH1 gene (NM_003118) was used as query sequences for the BLAST programs. The expression profiles for normal human tissues were obtained from GeneAnnot (26) and ArrayExpress (27). Northern analysis of NCBI's uniGene dataset was also extracted (21). Moreover, protein expression of HRH1 was obtained from SPIRE (28) and MOPED (29).

Meta-analysis of the prognostic value of the HRH1 gene in cancer. A database named 'PrognoScan' has been developed (30). This is i) a large collection of publicly available cancer microarray datasets with clinical annotation, as well as ii) a tool for assessing the biological relationship between gene expression and prognosis. PrognoScan employs the minimum P-value approach for grouping patients for survival analysis. PrognoScan provides a powerful platform for evaluating potential tumor markers and therapeutic targets and is publicly accessible at <http://gibk21.bio.kyutech.ac.jp/PrognoScan/index.html>. The human HRH1 gene was inputted as a query and the data were collected for analysis.

Results

Comparative proteomics of HRH1 proteins identified in vertebrate genomes. HRH1 genes were identified in the genome sequences of the human, chimpanzee, macaque, orangutan, dog, cow, horse, mouse, rat, opossum, chicken, *Xenopus tropicalis*, zebrafish and fugu. Refined phylogenetic trees using the identified HRH1 protein amino acid sequences by maximum likelihood (ML) and neighbor-joining (NJ) methods were almost identical (Fig. 1). We were unable to identify any site under positive selection with any of the six models in HRH1 proteins. Instead, the HRH1 proteins were under purifying selection (data not shown).

Expression profile of the human HRH1 gene. By EST sequence searching, the human HRH1 gene was found to be expressed in stomach, liver, heart, brain, placenta, embryonic tissue, kidney, vascular, intestine, esophagus, lung, adrenal gland, mouth, connective tissue, cervix, ovary, trachea, testis, uterus and skin. The investigation of available microarray experiments and 'virtual northern blot' showed a predominant expression of HRH1 in the bone marrow, whole blood, lymph node, thymus, brain, cerebellum, retina, spinal cord, heart, smooth muscle, skeletal muscle, small intestine, colon, adipocyte, kidney, liver, lung, pancreas, thyroid salivary gland, skin, ovary, uterus, placenta, prostate and testis. When searched in PrognoScan database, human HRH1 was also found to be expressed in bladder cancer, blood cancer, brain cancer, breast cancer, colorectal cancer, eye cancer, head and neck cancer, lung cancer, ovarian cancer, skin cancer and soft tissue cancer tissues. Among the protein expression databases SPIRE and MOPED, HRH1 protein was found to be only highly expressed in blood plasma.

Comparative genomics on the human HRH1 gene. Glucocorticoid receptor (GR), germ cell nuclear factor

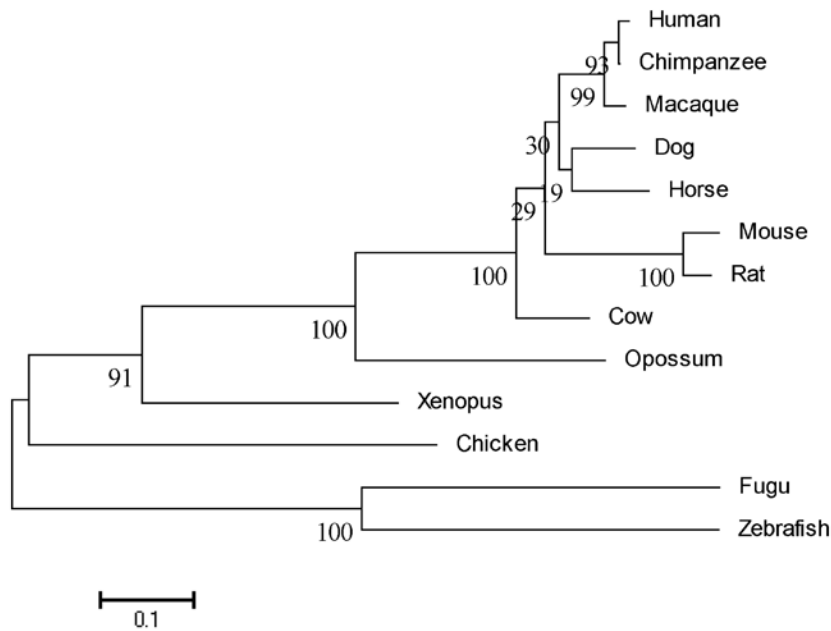


Figure 1. Phylogenetic analysis of HRH1. HRH1 genes were identified in the genome sequences of the human, chimpanzee, macaque, orangutan, dog, cow, horse, mouse, rat, opossum, chicken, *Xenopus tropicalis*, zebrafish and fugu. The phylogenetic tree of HRH1 gene was obtained by using maximum likelihood (ML) and neighbor-joining (NJ) methods. Primate HRH1 was clustered into one group, different from the other HRH1 genes.

(GCNF), signal transducer and activator of transcription 5A (STAT5A), mini Zinc finger 1 (MIF-1), heat shock transcription factor 2 (HSF2) and c-Myb regulatory transcription factor binding sites were identified in the HRH1 gene upstream (promoter) region.

Functional relevant SNP evaluation of the human HRH1 gene. Available SNPs (2,455) were identified in the human HRH1 gene. Among these, 88 SNPs were functionally relevant; including 4 available alleles disrupting an existing exonic splicing enhancer and 84 SNPs causing missense mutation (Table I).

Meta-analysis of the prognostic value of the human HRH1 gene in cancer. When given the gene, PrognosScan displays a summary in table format of tests for the gene with columns for dataset, cancer type, subtype, endpoint, cohort, contributor, array type, probe ID, number of patients, optimal cut-point, Pmin and Pcor. Among the databases which detected the expression of the HRH1 gene, 23 out of 153 tests showed an association between microarray expression in the HRH1 gene and cancer prognosis (bladder cancer 2/4, blood cancer 2/18, brain cancer 1/9, breast cancer 6/50, colorectal cancer 1/18, eye cancer 1/2, head and neck cancer 0/2, lung cancer 6/29, ovarian cancer 2/18, prostate cancer 0/1, skin cancer 0/2 and soft tissue cancer 2/2) with a 5% level of significance (Table II). Among the 6 breast cancer cases, low expression of the HRH1 gene was related to poor survival in 3 cases (GSE12276, GSE9893 and GSE1456-GPL96). However, high expression of the HRH1 gene was related to poor survival in another 3 cases of breast cancer (GSE6532-GPL570 and GSE3494-GPL96). As for the lung cancer cases, we found that high expression of the HRH1 gene was associated with poor survival in all 6 lung cancer cases including 5 adenocarcinoma cases and 1 non-small cell lung cancer case (GSE8894). Among the 2 blood cancer cases,

high expression of the HRH1 gene was related to poor survival in the B-cell lymphoma case (GSE4475). However, in 1 multiple myeloma case (GSE2658), low expression of the HRH1 gene was related to poor survival. We found that high expression of the HRH1 gene was associated with poor survival in 2 cases of soft tissue cancer and in 1 case of brain cancer and colorectal cancer. In the other cancer cases, low expression of the HRH1 gene was associated with poor survival in 2 cases of bladder cancer and ovarian cancer and 1 case of eye cancer.

Discussion

A large number of molecules involved in cell proliferation and key events in tumor development and progression have been extensively investigated including histamine (31-33). Histamine exerts its functions through binding to G protein-associated histamine H1, H2, H3, and H4 receptors (HRH1, HRH2, HRH3 and HRH4), resulting in the activation of different signal transduction pathways (31-33). In addition to the human HRH1, in the present study, we identified other HRH1 genes from other 13 vertebrate genomes and found that HRH1 exists in all types of vertebrates including the fish, amphibians, birds and mammals. All the identified HRH1 genes were encoding for a GPCR protein with a long third intracellular loop (IL3). The phylogenetic tree showed that HRH1 is separated for the order: fish, amphibians, birds and mammals. Primate HRH1s are almost the same and are clustered together. From the alignment and phylogenetic tree, mammalian HRH1s are conserved among the vertebrate genomes, suggesting that the function of HRH1 may have played an important physiological role for all vertebrates in the long process of evolution. Moreover, this process was under purifying selection, in accordance with the fact that multiple biological functions have been ascribed to this protein, including its function in central and peripheral tissues.

Table I. Functional relevant SNP evaluation of the human HRH1 gene.

SNP ID	Chr 3 position	Sequence	Type	AA change	SNP ID	Chr 3 position	Sequence	Type	AA change
rs79314450	11300765(+)	AAGATG/ATGTGA	Missense	I/M	rs199522994	11301463(+)	GGGGGA/TTGCCA	Missense	D/V
rs2067466	11300780(+)	AACAAG/CACCAC	Missense	N/K	rs199528452	11301826(+)	CACAGA/GGACAG	Missense	E/G
rs61738990	11300881(+)	CGTACG/AGAGTG	Missense	Q/R	rs200787735	11301096(+)	TTGATA/CGCTAC	Missense	S/R
rs189376051	11300922(+)	ACATCA/GTCAGC	Missense	I/V	rs199720564	11301138(+)	AGTATC/TGTACC	Missense	R/C
rs200571067	11300958(+)	GTGCCA/GTTCGTC	Missense	I/V	rs138669404	11300787(+)	CCACTA/GTGGCC	Missense	M/V
rs139664451	11301013(+)	GGGCCG/ATCCTC	Missense	H/R	rs111632894	11301870(+)	ACACAG/TGCCTG	Missense	GC
rs146441347	11301075(+)	TCAGTG/ATCTTC	Missense	I/V	rs148511138	11300908(+)	TGTGGG/AGAACC	Missense	/E/G
rs181783018	11301151(+)	GACCCA/GAGCCT	Missense	Q/R	rs200335907	11301073(+)	TTTCAA/GTGCTT	Missense	N/S
rs201947424	11301246(+)	CGGTGC/TGCCGA	Missense	R/C	rs201417298	11301732(+)	AGCAGA/GGCCTG	Missense	S/G
rs138120998	11301309(+)	CTGCCA/GTTCATC	Missense	I/V	rs201480303	11301124(+)	CCTCAG/TGTACC	Missense	R/M
rs201575633	11301362(+)	AAGATC/GTACAA	Missense	I/M	rs199843421	11300782(+)	CAAGAC/GCCTA	Missense	T/S
rs145938098	11301394(+)	GCACCG/AGGAGC	Missense	Q/R	rs201469326	11301462(+)	AGGGGA/GATGCC	Missense	ND
rs7651620	11301532(+)	TGGTGG/AATCTG	Missense	/E/G	rs201939588	11301699(+)	TCAAACC/TGGAGC	Missense	RW
rs201210547	11301661(+)	GGCTGC/TGGCAG	Missense	A/V	rs200553830	11301854(+)	TTGAGC/GAGTGG	Missense	S/R
rs149683927	11301678(+)	GTAGCA/GGGGAC	Missense	R/G	rs199720564	11301138(+)	AGTATC/TGTACC	Missense	R/C
rs147652731	11301693(+)	TAGCCG/ATCAAC	Missense	I/V	rs138669404	11300787(+)	CCACTA/GTGGCC	Missense	M/V
rs74840800	11301700(+)	CAAACCG/AGAGCC	Missense	Q/R	rs111632894	11301870(+)	ACACAG/TGCCTG	Missense	G/C
rs189287776	11301768(+)	CAGAGC/GATCAG	Missense	H/D	rs200695584	11300808(+)	TGATGC/TCCCTG	Missense	P/S
rs2067467	11301770(+)	GAGGAT/GCAGAT	Missense	/E/D	rs148511138	11300908(+)	TGTGGG/AGAACC	Missense	/E/G
rs141914966	11301810(+)	CGGACT/CCAGAT	Missense	P/S	rs201417298	11301732(+)	AGCAGA/GGCCTG	Missense	S/G
rs180831997	11301858(+)	GGAGTA/GGGTCT	Missense	R/G	rs201480303	11301124(+)	CCTCAG/TGTACC	Missense	R/M
rs199875751	11301988(+)	CATGGC/TAGCCT	Missense	A/V	rs199843421	11300782(+)	CAAGAC/GCCTA	Missense	T/S
rs2067470	11302069(+)	ACATTT/CGCACA	Missense	/S/L	rs201469326	11301462(+)	AGGGGA/GATGCC	Missense	N/D
rs76689102	11302092(+)	GGCTGG/TGCTAC	Missense	G/C	rs201939588	11301699(+)	TCAAACC/TGGAGC	Missense	R/W
rs202025055	11301685(+)	GGACTA/GTGTAG	Missense	Y/C	rs200553830	11301854(+)	TTGAGC/GAGTGG	Missense	S/R
rs149582378	11301513(+)	GGAAAGC/GCAAAA	Missense	P/A	rs200606524	11300890(+)	TGAGCA/GGAAGC	Missense	Q/R
rs201052869	11301024(+)	TCTGCC/GTCTTT	Missense	L/V	rs199954618	11301561(+)	AAACCC/GCCAAG	Missense	P/A
rs201175841	11301184(+)	GTTTCC/TCTCTT	Missense	P/L	rs202064669	11302032(+)	TCATGG/TTCAIT	Missense	V/F
rs201785749	11301523(+)	AGATGA/CTGGTG	Missense	D/A	rs200903220	11301886(+)	CATCAA/GGTTTA	Missense	K/R
rs201217782	11300932(+)	CCTCTC/TGGTGG	Missense	S/L	rs202100549	11302102(+)	CATCAA/GCTCCA	Missense	N/S
rs200086906	11301876(+)	GCCTGA/GAATAC	Missense	N/D	rs200892765	11302020(+)	ATTTCA/GTCTTC	Missense	I/V

Table I. Continued.

SNP ID	Chr 3 position	Sequence	Type	AA change	SNP ID	Chr 3 position	Sequence	Type	AA change
rs199638082	11301796(+)	ATCCTC/TCTCTC	Missense	S F	rs201450416	11301389(+)	TGCCAG/TCACCG	Missense	Q H
rs201053001	11301951(+)	ACCGCA/GAAAGG	Missense	K E	rs201101191	11301348(+)	TCTGGC/TTCTAT	Missense	L F
rs201586667	11301627(+)	TCTACA/TGCTTT	Missense	S C	rs201720863	11300901(+)	TCCACA/GCTGTG	Missense	T A
rs199822532	11300889(+)	GTGAGC/TGGAAG	Missense	R W	rs138501310	11301910(+)	CCGCTC/TGCAIT	Missense	S L
rs201386658	11301550(+)	GTCACC/TATCCC	Missense	P L	rs199645631	11300863(+)	CCTGCG/TGGTGC	Missense	R L
rs143882995	11300977(+)	GAACAT/CCCTCT	Missense	/T/I	rs200066638	11301247(+)	GGTGCA/GCCGAG	Missense	H R
rs200817909	11301139(+)	GTATCA/GTACCA	Missense	H R	rs201661278	11301150(+)	AGACCC/GGAGCC	Missense	R G
rs201903123	11301907(+)	GCTCCA/GCTCGC	Missense	H R	rs113526786	11300934(+)	TCTCGG/ATGGCG	Missense	/M/N
rs17855034	11301646(+)	TATTGA/TGCACA	Missense	E V	rs200526115	11302035(+)	TGGTCA/GTTGCC	Missense	I V
rs199824154	11301171(+)	TTCTGC/GGGGCC	Missense	R G	rs200897870	11301538(+)	ATCTGC/TCTTGA	Missense	A V
rs200547768	11301776(+)	CAGATG/TTTAGG	Missense	M I					

Available SNPs (2,455) were identified in the human HRH1 gene. Here, we show the 84 SNPs causing a missense mutation. AA change, amino acid change (slashes '/' indicate uncoded amino acids).

Histamine is an endogenous biogenic amine widely distributed throughout the organism and is long known to be a pleiotropic mediator in different (patho)-physiological conditions (1). Accordingly, by EST sequence search, one of its receptors, the HRH1 gene is also expressed in various tissues and cells including stomach, liver, heart, brain, placenta, embryonic tissue, kidney, vascular, intestine, esophagus, lung, adrenal gland, mouth, connective tissue, cervix, ovary, trachea, testis, uterus and skin. The same results were confirmed by the investigation of available microarray experiments and 'virtual northern blot', which showed a predominant expression of HRH1 in the bone marrow, whole blood, lymph node, thymus, brain, cerebellum, retina, spinal cord, heart, smooth muscle, skeletal muscle, small intestine, colon, adipocyte, kidney, liver, lung, pancreas, thyroid salivary gland, skin, ovary, uterus, placenta, prostate and testis. Available SNPs (2,455) were identified in the human HRH1 gene. We identified 88 SNPs including 4 available alleles disrupting an existing exonic splicing enhancer and 84 SNPs causing a missense mutation, which may impact the effect of histamine on the HRH1 protein. However, the effects of these SNPs on the physiological and pathological functions of HRH1 warrant further investigation.

Notably, most malignant cell lines and experimental tumors contain a high concentration of endogenous histamine, which can be released to the extracellular media via a paracrine or autocrine pathway. Moreover, histamine itself may be a crucial mediator in cancer development and progression by regulating diverse biological responses related to tumor growth including angiogenesis, cell invasion, migration, differentiation, apoptosis and modulation of the immune response (31-33). As one receptor of histamine, HRH1 expression in normal tissues vs. cancer samples has not been well studied. Following a search in the Prognoscan database, human HRH1 was also found to be expressed in bladder cancer, blood cancer, brain cancer, breast cancer, colorectal cancer, eye cancer, head and neck cancer, lung cancer, ovarian cancer, skin cancer and soft tissue cancer tissues. The expression of HRH1 in these tumors may explain the effects of histamine on cancer behaviors. Out of 153, 23 tests showed an association between microarray expression in the HRH1 gene and cancer prognosis (bladder cancer 2/4, blood cancer 2/18, brain cancer 1/9, breast cancer 6/50, colorectal cancer 1/18, eye cancer 1/2, head and neck cancer 0/2, lung cancer 6/29, ovarian cancer 2/18, prostate cancer 0/1, skin cancer 0/2 and soft tissue cancer 2/2) with a 5% level of significance. This suggests that the expression of HRH1 is related to the prognosis of many types of cancers including hematological and solid cancers. The mechanism of HRH1 involved in the process of these tumors warrants further investigation. It is important to note that the relationship between the expression of HRH1 and prognosis varied in different cancers, even in the same cancer from different databases. This implies that the function of HRH1 in these tumors may be multidimensional, not just as a tumor suppressor or oncogene.

The GR, GCNF, STAT5A, MIF-1, HSF2 and c-Myb regulatory transcription factor binding sites were identified in the HRH1 gene upstream (promoter) region. GRs are nuclear hormone receptors of the NR3C class, which also includes mineralocorticoid, progesterone and androgen receptors. GRs are ubiquitously expressed and mediate a vast array of physiological functions. Glucocorticoids are widely

Table II. Dataset content from PrognosScan indicated an association between microarray expression of HRH1 and cancer prognosis.

Database	Case type	Subtype	No. of patients	Endpoint	Cutpoint	P-value	Prognosis	Refs.
GSE13507	Bladder cancer		165	Overall survival	0.25	0.012	1	(41,42)
GSE13507	Bladder cancer	Transitional cell carcinoma	165	Disease-specific survival	0.28	0.009	1	(41,42)
GSE4475	Blood cancer	B-cell lymphoma	158	Overall survival	0.76	0.027	2	(43)
GSE2658	Blood cancer	Multiple myeloma	559	Disease-specific survival	0.67	0.002	1	(44)
GSE4271-GPL96	Brain cancer	Astrocytoma	77	Overall survival	0.39	0.04	2	(45)
GSE12276	Breast cancer		204	Relapse-free survival	0.88	0.047	1	(46)
GSE6532-GPL570	Breast cancer		87	Distant metastasis-free survival	0.86	0.01	2	(47)
GSE6532-GPL570	Breast cancer		87	Relapse-free survival	0.86	0.01	2	(47)
GSE9893	Breast cancer		155	Overall survival	0.29	0.012	1	(48)
GSE1456-GPL96	Breast cancer		159	Relapse-free survival	0.11	0.007	1	(49)
GSE3494-GPL96	Breast cancer		249	Relapse-free survival	0.8	0.0014	2	(50)
GSE17536	Colorectal cancer		145	Relapse-free survival	0.9	0.043	2	(51)
GSE22138	Eye cancer	Uveal melanoma	63	Relapse-free survival	0.22	0.017	1	(52)
jacob-00182-CANDF	Lung cancer	Adenocarcinoma	82	Relapse-free survival	0.77	0.047	2	(53)
GSE13213	Lung cancer	Adenocarcinoma	117	Relapse-free survival	0.55	0.046	2	(54)
GSE31210	Lung cancer	Adenocarcinoma	204	Relapse-free survival	0.68	0.03	2	(55)
GSE31210	Lung cancer	Adenocarcinoma	204	Relapse-free survival	0.85	0.018	2	(55)
jacob-00182-UM	Lung cancer	Adenocarcinoma	178	Relapse-free survival	0.68	0.02	2	(53)
GSE8894	Lung cancer	Non-small cell lung cancer	138	Relapse-free survival	0.78	0.036	2	(56)
GSE17260	Ovarian cancer		110	Relapse-free survival	0.88	0.048	1	(57)
GSE14764	Ovarian cancer		80	Relapse-free survival	0.12	0.01	1	(57)
GSE30929	Soft tissue cancer	Liposarcoma	140	Relapse-free survival	0.86	0.001	2	(58)
GSE30929	Soft tissue cancer	Liposarcoma	140	Relapse-free survival	0.88	0.03	2	(58)

Out of 153 tests, 23 showed an association between microarray expression of HRH1 gene and cancer prognosis (bladder cancer 2/4, blood cancer 2/18, brain cancer 1/9, breast cancer 6/50, colorectal cancer 1/18, eye cancer 1/2, head and neck cancer 0/2, lung cancer 6/29, ovarian cancer 2/18, prostate cancer 0/1, skin cancer 0/2 and soft tissue cancer 2/2) with a 5% significance level.

used for the treatment of lymphoid malignancy due to their marked effects on cell cycle progression and apoptosis (34). Glucocorticoids are able to alter signaling in key survival pathways and this can result in reversible growth arrest or cell death in certain cell types (34-36). STAT5A is a member of the STAT family of transcription factors. It is activated by, and mediates the responses of many cell ligands, such as IL2, IL3, IL7, GM-CSF, erythropoietin, thrombopoietin and different growth hormones. Activation of this protein in many types of cancers has been shown to be essential for tumorigenesis (37,38). C-Myb encodes a transcription factor that regulates the expression of numerous genes during cell cycle progression. c-Myb is a cellular proto-oncogene, which has the ability to regulate the expression of cell cycle genes and is involved in cell proliferation and carcinogenesis (39,40). These tumor-related transcriptional factors may be involved in the effect of HRH1 in tumors.

Acknowledgements

This study was sponsored by the Chinese National Natural Science Foundation (81372828, 81302331, 810001329), The Talent Foundation of The First Affiliated Yijishan Hospital of Wannan Medical College (YR201305), the Natural Science Foundation of Guangdong Province (no. 10151503102000045), the National Major Scientific and Technological Special Project for 'Significant New Drug Development' (2011ZX09302-003-02), the Jiangsu Province Major Scientific and Technological Special Project (BM2011017), and a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References

- Seifert R, Strasser A, Schneider EH, Neumann D, Dove S and Buschauer A: Molecular and cellular analysis of human histamine receptor subtypes. *Trends Pharmacol Sci* 34: 33-58, 2013.
- Yoshizawa M, Tashiro M, Fukudo S, *et al*: Increased brain histamine H1 receptor binding in patients with anorexia nervosa. *Biol Psychiatry* 65: 329-335, 2009.
- Booth RG, Fang L, Wilczynski A, *et al*: Molecular determinants of ligand-directed signaling for the histamine H1 receptor. *Inflamm Res* 57 (Suppl 1): S43-S44, 2008.
- Swan C, Richards SA, Duroudier NP, Sayers I and Hall IP: Alternative promoter use and splice variation in the human histamine H1 receptor gene. *Am J Respir Cell Mol Biol* 35: 118-226, 2006.
- Oda T, Morikawa N, Saito Y, Masuho Y and Matsumoto S: Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem* 275: 36781-36788, 2000.
- Breunig E, Michel K, Zeller F, Seidl S, Weyhern CW and Schemann M: Histamine excites neurones in the human submucous plexus through activation of H1, H2, H3 and H4 receptors. *J Physiol* 583: 731-742, 2007.
- Brew OB and Sullivan MH: Localisation of mRNAs for diamine oxidase and histamine receptors H1 and H2, at the feto-maternal interface of human pregnancy. *Inflamm Res* 50: 449-452, 2001.
- Arisawa T, Tahara T, Ozaki K, *et al*: Association between common genetic variant of *HRH2* and gastric cancer risk. *Int J Oncol* 41: 497-503, 2012.
- Lefranc F, Yeaton P, Brotchi J and Kiss R: Cimetidine, an unexpected anti-tumor agent, and its potential for the treatment of glioblastoma. *Int J Oncol* 28: 1021-1030, 2006.
- Davio C, Baldi A, Mladovan A, Cricco G, Fitzsimons C and Bergoc RR: Expression of histamine receptors in different cell lines derived from mammary gland and human breast carcinomas. *Inflamm Res* 44 (Suppl 1): S70-S71, 1995.
- Jiang CG, Liu FR, Yu M, Li JB and Xu HM: Cimetidine induces apoptosis in gastric cancer cells *in vitro* and inhibits tumor growth *in vivo*. *Oncol Rep* 23: 693-700, 2010.
- Fukuda M, Tanaka S, Suzuki S, Kusama K, Kaneko T and Sakashita H: Cimetidine induces apoptosis of human salivary gland tumor cells. *Oncol Rep* 17: 673-678, 2007.
- Yang L, Luo Y and Wei J: Integrative genomic analyses on *Ikaros* and its expression related to solid cancer prognosis. *Oncol Rep* 24: 571-577, 2010.
- Yang L, Luo Y, Wei J and He S: Integrative genomic analyses on IL28RA, the common receptor of interferon- λ 1, - λ 2 and - λ 3. *Int J Mol Med* 25: 807-812, 2010.
- Yang L, Wei J and He S: Integrative genomic analyses on interferon- λ s and their roles in cancer prediction. *Int J Mol Med* 25: 299-304, 2010.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG: The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 15: 4876-4882, 1997.
- Guindon S, Lethiec F, Duroux P and Gascuel O: PHYML Online - a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* 33: W557-W559, 2005.
- Kumar S, Tamura K and Nei M: MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5: 150-163, 2004.
- Yang Z: PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput Appl Biosci* 13: 555-556, 1997.
- Yang Z, Nielsen R, Goldman N and Pedersen AM: Codon-substitution models for heterogeneous selection pressure at amino acid sites. *Genetics* 155: 431-449, 2000.
- Yu H, Yuan J, Xiao C and Qin Y: Integrative genomic analyses of receptor d'origine nantais and its prognostic value in cancer. *Int J Mol Med* 31: 1248-1254, 2013.
- Katoh Y and Katoh M: Integrative genomic analyses on *GLII*: positive regulation of GLI1 by Hedgehog-GLI, TGF β -Smads, and RTK-PI3K-AKT signals, and negative regulation of GLI1 by Notch-CSL-HES/HEY, and GPCR-Gs-PKA signals. *Int J Oncol* 35: 187-192, 2009.
- Katoh M and Katoh M: Integrative genomic analyses of *WNT11*: transcriptional mechanisms based on canonical WNT signals and GATA transcription factors signaling. *Int J Mol Med* 24: 247-251, 2009.
- Katoh M and Katoh M: Transcriptional mechanisms of *WNT5A* based on NF- κ B, Hedgehog, TGF β , and Notch signaling cascades. *Int J Mol Med* 23: 763-769, 2009.
- Katoh M and Katoh M: Integrative genomic analyses of ZEB2: Transcriptional regulation of ZEB2 based on SMADs, ETS1, HIF1 α , POU/OCT, and NF- κ B. *Int J Oncol* 34: 1737-1742, 2009.
- Chalifa-Caspi V, Yanai I, Ophir R, *et al*: GeneAnnot: comprehensive two-way linking between oligonucleotide array probesets and GeneCards genes. *Bioinformatics* 20: 1457-1458, 2004.
- Parkinson H, Sarkans U, Shojatalab M, *et al*: ArrayExpress - a public repository for microarray gene expression data at the EBI. *Nucleic Acids Res* 33: D553-D555, 2005.
- Kolker E, Higdon R, Morgan P, *et al*: SPIRE: Systematic protein investigative research environment. *J Proteomics* 75: 122-126, 2011.
- Kolker E, Higdon R, Haynes W, *et al*: MOPED: Model Organism Protein Expression Database. *Nucleic Acids Res* 40: D1093-D1099, 2012.
- Mizuno H, Kitada K, Nakai K and Sarai A: PrognScan: a new database for meta-analysis of the prognostic value of genes. *BMC Med Genomics* 2: 18, 2009.
- Xu TR, Yang Y, Ward R, Gao L and Liu Y: Orexin receptors: multi-functional therapeutic targets for sleeping disorders, eating disorders, drug addiction, cancers and other physiological disorders. *Cell Signal* 25: 2413-2423, 2013.
- Medina VA and Rivera ES: Histamine receptors and cancer pharmacology. *Br J Pharmacol* 61: 755-767, 2010.
- Blaya B, Nicolau-Galmés F, Jangi SM, *et al*: Histamine and histamine receptor antagonists in cancer biology. *Inflamm Allergy Drug Targets* 9: 146-157, 2010.
- Schlossmacher G, Stevens A and White A: Glucocorticoid receptor-mediated apoptosis: mechanisms of resistance in cancer cells. *J Endocrinol* 211: 17-25, 2011.
- Feng Y, Bai X, Yang Q, Wu H and Wang D: Downregulation of 15-lipoxygenase 2 by glucocorticoid receptor in prostate cancer cells. *Int J Oncol* 36: 1541-1549, 2010.

36. Jang JH, Woo SM, Um HJ, *et al*: RU486, a glucocorticoid receptor antagonist, induces apoptosis in U937 human lymphoma cells through reduction in mitochondrial membrane potential and activation of p38 MAPK. *Oncol Rep* 30: 506-512, 2013.
37. Koptyra M, Gupta S, Talati P and Nevalainen MT: Signal transducer and activator of transcription 5a/b: biomarker and therapeutic target in prostate and breast cancer. *Int J Biochem Cell Biol* 43: 1417-1421, 2011.
38. Hou L, Xu B, Mohankumar KM, Goffin V, Perry JK, Lobie PE and Liu DX: The prolactin receptor mediates HOXA1-stimulated oncogenicity in mammary carcinoma cells. *Int J Oncol* 41: 2285-2295, 2012.
39. Haeri M, Li Y, Li Q, Spaner DE and Ben-David Y: Insertional activation of myb by F-MuLV in SCID mice induces myeloid leukemia. *Int J Oncol* 43: 169-176, 2013.
40. Zhang J, Luo N, Luo Y, Peng Z, Zhang T and Li S: microRNA-150 inhibits human CD133-positive liver cancer stem cells through negative regulation of the transcription factor c-Myb. *Int J Oncol* 40: 747-756, 2012.
41. Kim WJ, Kim EJ, Kim SK, *et al*: Predictive value of progression-related gene classifier in primary non-muscle invasive bladder cancer. *Mol Cancer* 9: 3, 2010.
42. Lee JS, Leem SH, Lee SY, *et al*: Expression signature of E2F1 and its associated genes predict superficial to invasive progression of bladder tumors. *J Clin Oncol* 28: 2660-2667, 2010.
43. Hummel M, Bentink S, Berger H, *et al*: Molecular Mechanisms in Malignant Lymphomas Network Project of the Deutsche Krebshilfe. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. *N Engl J Med* 354: 2419-2430, 2006.
44. Zhan F, Huang Y, Colla S, *et al*: The molecular classification of multiple myeloma. *Blood* 108: 2020-2028, 2006.
45. Phillips HS, Kharbanda S, Chen R, *et al*: Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 9: 157-173, 2006.
46. Bos PD, Zhang XH, Nadal C, *et al*: Genes that mediate breast cancer metastasis to the brain. *Nature* 459: 1005-1009, 2009.
47. Loi S, Haibe-Kains B, Majjaj S, *et al*: PIK3CA mutations associated with gene signature of low mTORC1 signaling and better outcomes in estrogen receptor-positive breast cancer. *Proc Natl Acad Sci USA* 107: 10208-10213, 2010.
48. Chanrion M, Negre V, Fontaine H, *et al*: A gene expression signature that can predict the recurrence of tamoxifen-treated primary breast cancer. *Clin Cancer Res* 14: 1744-1752, 2008.
49. Pawitan Y, Bjöhle J, Amler L, *et al*: Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. *Breast Cancer Res* 7: R953-R964, 2005.
50. Miller LD, Smeds J, George J, *et al*: An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci USA* 102: 13550-13555, 2005.
51. Smith JJ, Deane NG, Wu F, *et al*: Experimentally derived metastasis gene expression profile predicts recurrence and death in patients with colon cancer. *Gastroenterology* 138: 958-968, 2010.
52. Laurent C, Valet F, Planque N, *et al*: High PTP4A3 phosphatase expression correlates with metastatic risk in uveal melanoma patients. *Cancer Res* 71: 666-674, 2011.
53. Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma, Shedden K, Taylor JM, *et al*: Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. *Nat Med* 14: 822-827, 2008.
54. Tomida S, Takeuchi T, Shimada Y, *et al*: Relapse-related molecular signature in lung adenocarcinomas identifies patients with dismal prognosis. *J Clin Oncol* 27: 2793-2799, 2009.
55. Okayama H, Kohno T, Ishii Y, *et al*: Identification of genes upregulated in ALK-positive and EGFR/KRAS/ALK-negative lung adenocarcinomas. *Cancer Res* 72: 100-111, 2012.
56. Lee ES, Son DS, Kim SH, *et al*: Prediction of recurrence-free survival in postoperative non-small cell lung cancer patients by using an integrated model of clinical information and gene expression. *Clin Cancer Res* 14: 7397-7404, 2008.
57. Yoshihara K, Tajima A, Yahata T, *et al*: Gene expression profile for predicting survival in advanced-stage serous ovarian cancer across two independent datasets. *PLoS One* 5: e9615, 2010.
58. Gobble RM, Qin LX, Brill ER, *et al*: Expression profiling of liposarcoma yields a multigene predictor of patient outcome and identifies genes that contribute to liposarcomagenesis. *Cancer Res* 71: 2697-2705, 2011.