# *HAVcR-1* Expression in Human Colorectal Cancer and its Effects on Colorectal Cancer Cells *In Vitro*

YOUXIN WANG[1,2,3], TRACEY A MARTIN[1,3*] and WEN G. JIANG[1,3]

[1]*Metastasis and Angiogenesis Research Group, Institute of Cancer and Genetics, Cardiff University School of Medicine, Cardiff, U.K.;*
[2]*School of Public Health and Family Medicine, Capital Medical University, Beijing, China;*
[3]*Cardiff University-Capital Medical University Joint Centre for Biomedical Research, School of Basic Medical Science, Capital Medical University, Beijing, China*

**Abstract.** *Background: Hepatitis A virus cellular receptor 1 (HAVcR-1) may function as part of the regulatory apparatus for tight junction in human endothelial cells, and is overexpressed in renal cell and ovarian clear cell carcinomas. Materials and Methods: HAVcR-1 expression in colorectal cancer and matched background tissues was determined using quantitative-PCR. HAVcR-1 was overexpressed in three colorectal cancer cell lines and the effect of this overexpression was assessed by changes in cell behaviour. Results: HAVcR-1 expression was increased in colorectal cancer ($p<0.001$) and the disease-free time of patients with high levels of HAVcR-1 expression was significantly longer ($p=0.045$). HAVcR-1 overexpression reduced colorectal cancer cell adhesion and invasion ($p<0.016$). Conclusion: HAVcR-1 overexpression may be a diagnostic biomarker for colorectal cancer and a prognostic marker for a longer disease-free interval after surgery for colorectal cancer. HAVcR-1 overexpression can reduce cell adhesion and invasion and could thus be involved in the metastatic cascade.*

Colorectal cancer is the third most commonly diagnosed cancer in the world and is more common in developed countries (1). It is estimated that worldwide, in 2008, 1.23 million new cases of colorectal cancer were clinically diagnosed, resulting in 608,000 deaths (1). Colorectal cancer develops as a result of progressive accumulation of genetic and epigenetic alterations, leading to genetic instability ultimately resulting in malignant transformation (2). Therefore, understanding the molecular basis of individual susceptibility to colorectal cancer and determining factors that initiate the development of the tumour and drive its progression is of great importance.

Hepatitis A virus cellular receptor-1 (*HAVcR-1*), previously found to be located on mouse chromosome 11 and human chromosome 5q31-33, is associated with disease susceptibility (3, 4). *HAVcR-1*, also known as human kidney injury molecule-1 (KIM-1) and T-cell immunoglobulin and mucin domain-1 (Tim-1), is the cellular receptor for *hepatotropic picornavirus*, which causes acute hepatitis A in humans (5, 6).

*HAVcR-1* is also a type-I cell membrane glycoprotein with an extracelluar domain containing N-terminal cysteine-rich regions and an extended *O*-glycosylated threonine-, serine- and praline-rich regions which interact with extracellular molecules. Previously, we showed that *HAVcR-1* might have a novel function as part of the regulatory apparatus for tight junctions (TJs) in human endothelial cells (7). TJs are well-known to function as a control for the paracellular diffusion of ions and certain molecules, it has however, become evident that TJs have a vital role in maintaining cell-to-cell integrity. Loss of cohesion of the TJ structure can lead to invasion and ultimately to metastasis of cancer cells (8). In addition, it is reported that *HAVcR-1* is overexpressed in renal cell carcinoma and ovarian clear cell carcinoma (9-11). Consequently, *HAVcR-1* might serve as a candidate gene associated with cancer susceptibility, tumour development and progression.

In this study, we aimed to explore the association between *HAVcR-1* expression and colorectal cancer (incidence and behaviour), and to investigate the molecular mechanisms accounting for any association.

## Materials and Methods

*Cell lines.* The cell lines used were the human colorectal cancer cell lines HRT-18, CaCO2 and HT115, which were purchased from the European Collection of Cell Culture (ECACC, Porton Down, UK). Cells were routinely cultured with Dulbecco's modified Eagle's

*Correspondence to:* Dr. Tracey A. Martin, Metastasis and Angiogenesis Research Group, Institute of Cancer and Genetics, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, U.K. Tel: +44 292074 6536, Fax: +44 2920762896, e-mail: martinta1@cardiff.ac.uk

*Key Words:* HAVcR-1, hepatitis A virus cellular receptor-1, colorectal cancer, prognosis, invasion.

medium (DMEM; Sigma-Aldrich Company Ltd. Poole, Dorset, UK) supplemented with 10% fetal calf serum (FCS), penicillin and streptomycin (GIBO BRL, Paisley, UK). RNA extraction kit and reverse transcription (RT) kit were obtained from AbGene (Guildford, Surrey, UK).

*Specimens*. Freshly-frozen colorectal cancer tissues (n=84), along with matched normal tissue from the same patients (n=54), were obtained from patients who attended the University Hospital of Cardiff University from January 2003 to December 2009. In addition, normal colorectal tissues (n=64) served as control. Both normal tissues and cancer tissues were examined and confirmed by pathologists. All protocols were reviewed and approved by the local Ethical Committee (NRES 05/wse03/92 and 05/DMD/3562) and all patients gave written informed consent. Histological types of colorectal cancer are given in Table I. Clinicopathological factors, including age, sex, histological type of tumours, TNM stage, and lymph node metastasis were recorded and stored in the patients' database. Patients were followed-up from the day of operation to December 2009, as the end of the follow-up for the present study. The follow-up intervals were calculated as survival intervals after surgery.

*Plasmids and transfection*. An *HAVcR-1* expression plasmid has been previously contructed using the pEF6/V5-His-TOPO plasmid vector with *HAVcR-1* full reading frame (7). The empty plasmid control (PEF) and *HAVcR-1* expression plasmid (HAVcR1) were transfected into HT115, HRT18 and CaCO2 cells, respectively, using an electroporator (Easject Plus; EquiBio, Ltd., Kent, UK). Stable transfectants were obtained and verified after three weeks of selection using blasticidin.

*RNA isolation, reverse transcription PCR and quantitative (QPCR) real-time PCR*. *T*otal RNA was obtained using TRI Reagent (Sigma-Aldrich, Inc., Poole, D0rset, UK). First-strand cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR was performed using REDTaq ReadyMix PCR reaction. Cycling conditions were 94˚C for 5 min, followed by 36 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 40 s. This was followed by a final extension of 10 min at 72˚C. The products were visualized in 2% agarose gels stained with ethidium bromide. Real-time quantitative PCR (qPCR) was performed on the Icycler IQ5 system (Bio-Rad, Hammel Hemstead, UK) to quantify the level of *HAVcR-1* transcripts in the colorectal carcinoma specimens (shown as copies/μl from internal standard). The QPCR technique utilized the Amplifluor system™ (Intergen Inc. Watford, London, England) (12) and the QPCR master mix (Bio-Rad). The pairs of primers used are were listed in Table II. Real-time QPCR conditions were 95˚C for 15 min, followed by 60 cycles at 95˚C for 20 s, 55˚C for 30 s and 72˚C for 20 s. QPCR for Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was also performed on the same samples to normalize for any residual differences in the initial level of RNA in the specimens.

*In vitro cell growth assay*. A standard procedure was used as previously described (13). Cells were plated into a 96-well plate (2,500 cells/well). Cell growth was assessed after 1, 3, and 5 days. Crystal violet was used to stain cells, and the absorbance was determined at a wavelength of 540 nm using a spectrophotometer (Elx800; Bio-Tek, Bedford, Bedfordshire, UK).

Table I. *Clinical data of the patients with colorectal cancer.*

| Clinical data | | Sample size |
|---|---|---|
| Tissue sample | Tumour | 83 |
| | matched normal tissue | 54 |
| Differentiation status[1] | 1 | 2 |
| | 2 | 46 |
| | 3 | 14 |
| Nodal status | 0 | 34 |
| | 1 | 15 |
| | 2 | 13 |
| TNM status | 1 | 9 |
| | 2A | 22 |
| | 2B | 3 |
| | 3A | 2 |
| | 3B | 11 |
| | 3C | 10 |
| Duke's status | A | 7 |
| | B | 28 |
| | C | 29 |
| Location | Left colon | 22 |
| | Right colon | 24 |
| | Trans colon | 2 |
| | Rectum | 18 |
| Invasion status | No invasion | 43 |
| | Invasion | 25 |

[1]According to pathological evaluation.

*In vitro invasion assay*. Transwell inserts with 8 nm pore sizes were coated with 50 μg Matrigel and air-dried (BD Matrigel Basement Membrane Matrix, Cat Number 354234; BD Bio-Science, Oxford, UK). The Matrigel was rehydrated before use. A total of 25,000 cells were added to each well and after 96 h, cells that had migrated through the matrix and pores were fixed (4% formalin), stained in crystal violet, and counted.

*Cell-matrix adhesion assay*. A total of 40,000 cells were added to each well of a 96-well plate previously prepared by coating with Matrigel (5 μg/well). The cells were incubated at 37˚C in 5% $CO_2$ for 40 min and the medium was then discarded. Non-adherent cells were washed-off using buffered saline solution (BSS). The remaining cells were then fixed in 4% formaldehyde for 5 min. After further washing, cells were stained with crystal violet, and the number of adherent cells was then counted.

*Wounding/migration assay*. The wounding assay was performed as previously described (13). The cells were seeded at a density of 40,000 per well into a 24-well plate and allowed to reach confluence. The monolayer of cells was then scraped with a fine gauge needle to create a wound of approximately 200 μm. The movement of cells to close the wound was recorded as previously described using a time-lapsed video system (13). Images were captured from the videotape at the equivalent of 15 min intervals in real time and stored as a series of gray scale bitmap images. The movement of single cells within a colony was analyzed by tracking each cell's boundary, for each frame in a series, using Optimas 6.0 motion analysis (Meyer Instruments, Houston, TX, USA).

Table II. *PCR primer sequences used in this study.*

| | Sense (5'-3') | Antisense (5'-3') |
|---|---|---|
| Hepatocyte A virus cellular receptor 1 (*HAVcR1*) (RT-PCR) | atgcatcctcaagtggtc | accccaatcttcatgtccacatt |
| Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (RT-PCR) | atgatatcgcgccgctcgtc | gctcggtcaggatcttca |

*Statistical analysis*. Statistical analysis was performed using the SPSS software (SPSS Standard version 13.0; SPSS Inc. IBM. London, UK). The relationship between *HAVcR-1* expression and tumour grade, TNM staging and nodal status was assessed by Mann Whitney *U*-test. The error bars shown in the graphs represent the 95% confidence intervals (CI). Survival curves were analyzed using Kaplan Meier survival analysis. Differences were considered statistically significant at $p<0.05$, and all the tests were two-sided.

## Results

*The expression pattern of HAVcR-1 in colorectal cancer tissues*. HAVcR-1 transcript expression was examined in tumour specimens from patients with colorectal cancer (n=84, amongst them 54 specimens with matched normal colon tissue) and normal control using real-time quantitative PCR (expressed as mean HAVcR-1 transcript copies/μl of RNA from 50 ng total RNA and standardized with *GAPDH*). HAVcR-1 was significantly overexpressed ($p<0.001$, MannWhitney *U*-test) in tumour tissues (median=10.0, Q1~Q3=1~330), compared with normal tissues (median=0, Q1-Q3=0-2.4), and also significantly overexpressed (p<0.001, MannWhitney U test) when compared to the self-matched normal colonic tissues (median=0, Q1-Q3=0-2.6) (Figure 1).

The distribution of invasion status, treatment methods, location of cancer tissues, Dukes type, TNM type, node status and differentiation status were not associated with HAVcR-1 expression (data not shown). Therefore, in the survival analysis, Kaplan Meier analysis was used, not the Cox regression model.

*The prognostic significance of HAVcR-1 expression*. In order to explore the prognostic significance of HAVcR-1 expression in colorectal cancer, we defined the expression of more than the mean copy number plus two standard deviations as overexpression (3.28+2x137.0=306.8).

Under this definition, HAVcR-1 was overexpressed in 22 samples of colorectal cancer (26.5%, 95% CI=17.0%-36.0%).

Kaplan Meier analysis demonstrated that overall time-to-recurrence was not correlated with HAVcR-1 expression ($p=0.08$, no statistics were computed because the majority of cases were censored), and overall time-to-metastasis was not significantly correlated with HAVcR-1 expression (mean 142.5 weeks, 95% CI=107.6-177.4, and 125.3 weeks, 95% CI=102.8-147.9 weeks in patients with high expression and low expression of HAVcR-1, respectively) ($p= 0.496$, Figure 1D). The overall survival time after surgery in patients with high levels of HAVcR-1 expression was 150.2 weeks (95% CI=122.5-178.0 weeks), while the lower expression levels-group it was 106.1 weeks ($p= 0.076$, Figure 1E). The disease-free interval for patients with high levels of HAVcR-1 expression (mean 151.6 weeks, 95% CI=119.2-184.1 weeks) was significantly longer than that of patients with a lower expression (mean 85.7 weeks, 95% CI=48.0-123.3 weeks) ($p=0.045$, Figure 1F).

*Stable HAVcR-1 overexpression in cancer cell lines*. HAVcR-1 was not expressed in three colorectal cancer cells, HRT18, CaCO2 and HT115, therefore we transfected the HAVcR-1 expression plasmids to these cells and determined the expression of HAVcR-1 by RT-PCR. Figure 2 shows HAVcR-1 to be overexpressed in HRT18, CaCO2 and HT115 cells following transfection with the HAVcR-1 expression plasmid, compared to those of wild-type and empty plasmids (PEF).

*Effect of HAVcR-1 overexpression on cell growth, adhesion, migration and invasion*. There was no significant influence on the *in vitro* growth of cells after the overexpression of HAVcR-1, either in HRT18, CaCO2 or HT115 cells, compared with their controls (Figure 3A-C). A lower number of cells adhering to matrix was observed in the three colorectal cancer cell lines when HAVcR-1 was overexpressed compared with the respective control, $p<0.001$, $p=0.009$ and $p=0.026$ in HRT18, CaCO2 and HT115 cells, respectively (Figure 3D-F). Overexpression of HAVcR-1 did not affect the motile nature of three colorectal cancer cells (Figure 3G-I). Figure 3J-L shows that overexpression of HAVcR-1 was associated with the reduction of cancer cell invasion in all three colorectal cancer cells ($p<0.001$, $p<0.001$, and $p=0.016$ in HRT18, CaCO2 and HT115 cells, respectively).

## Discussion

HAVcR-1 has multiple functions and is associated with some diseases, such as autoimmune diseases (asthma, allergy, systemic lupus erythematosus patients, rheumatoid arthritis) (14, 15-18), kidney injury (19, 20), childhood atopic dermatitis (21), cancer (clear cell renal cell carcinoma, ovarian clear cell carcinoma) (9-11) and infection (cerebral malaria, hepatitis C virus, hepatitis A virus, human immunodeficiency virus, zaire ebolavirus, lake victoria marburgvirus and *Helicobacter pylori*) (22-27).

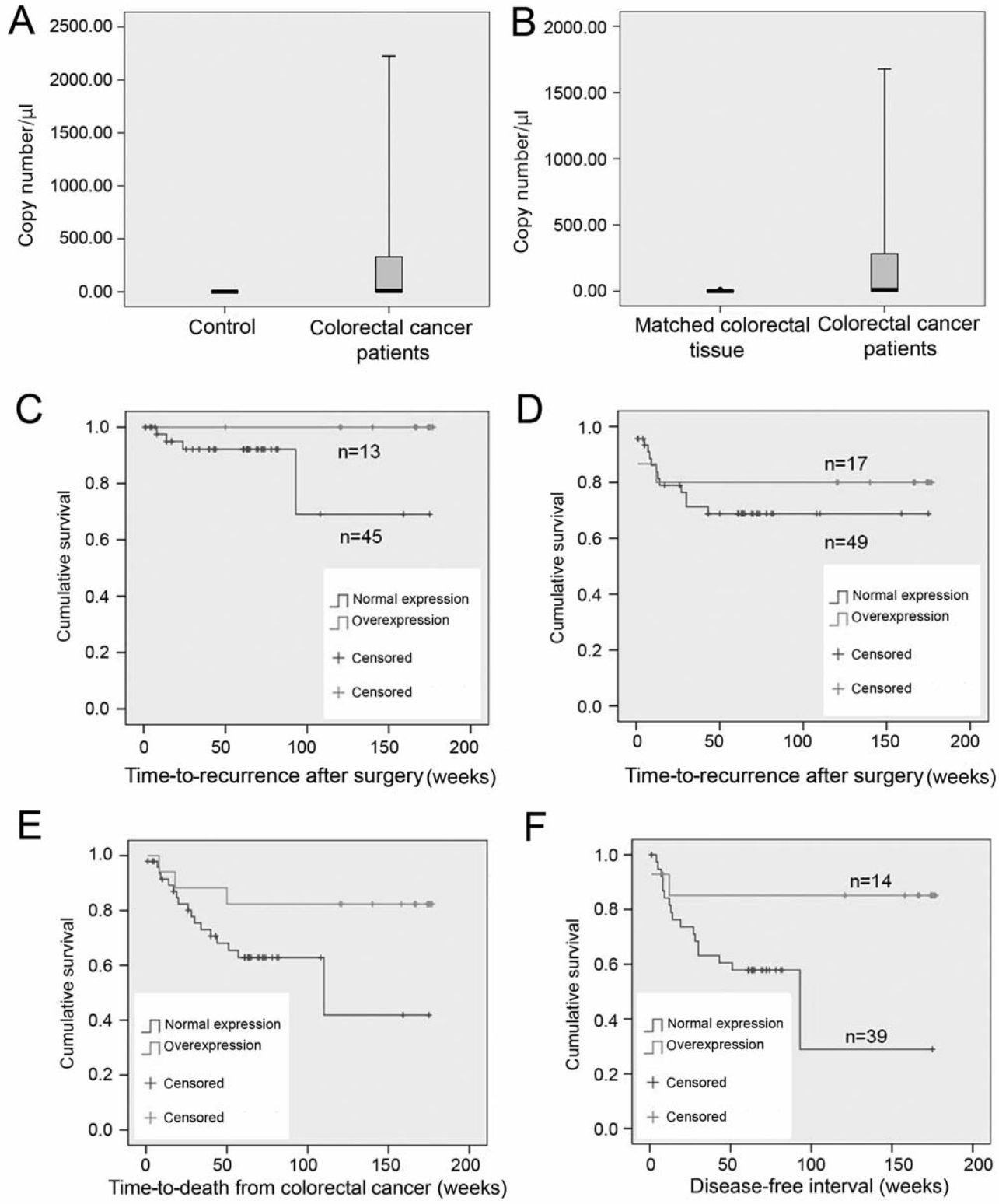


Figure 1. *Quantitative polymerase chain reaction (PCR) analysis of hepatocyte A virus cellular receptor-1 (HAVcR-1) expression in human colorectal cancer tissues. A: Tumour versus normal colorectal tissues of healthy individuals. B: Tumour versus matched normal colorectal tissues of the patients. Overall survival analysis of time-to-recurrence (C), time-to-metastasis (D), time-to-death due to colorectal cancer (E) and disease-free interval (F), after surgery for patients with colorectal cancer with high or low HAVcR-1 expression.*

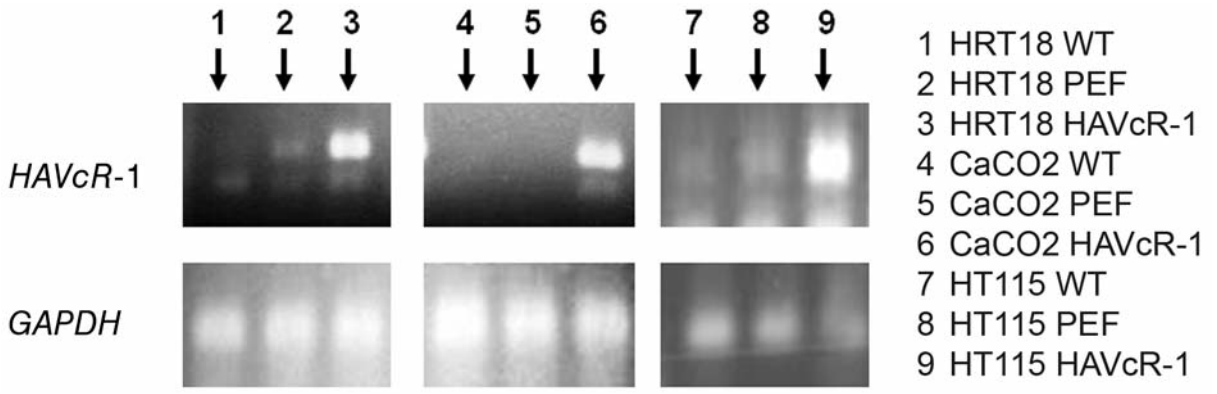


Figure 2. *Hepatocyte A virus cellular receptor 1 (HAVcR-1) mRNA expression in cancer cell lines. WT: Wild type, PEF: empty pEF6/V5-His-TOPO plasmid, HAVcR-1: HAVcR-1 expression plasmid (in pEF6/V5-His-TOPO plasmid).*

In this study, we demonstrated that HAVcR-1 is overexpressed in colorectal cancer ($p<0.001$), which is consistent with data published for clear cell renal cell carcinoma (9-11). Two case control studies suggested that HAVcR-1 is a useful biomarker for diagnosing renal cell carcinoma and ovarian clear cell carcinoma (11). The present study supports this conclusion, in that HAVcR-1 may be a useful biomarker for diagnosing colorectal cancer.

Two possibilities might explain the association between HAVcR-1 overexpression and tumourigenesis: It has been shown that HAVcR-1 itself may be able to block cell differentiation, as shown by its overexpression in 769-P cells (9). Alternatively, it is also suggested that a high expression level of HAVcR-1 can increase the incidence of infection by *Helicobacter* (28), HIV (29), *etc*., thus promoting susceptibility to colorectal cancer.

We also demonstrated that the disease-free interval (after surgery) in patients with high levels of HAVcR-1 expression was significantly longer than that for those with a lower expression. In addition, overall time-to-recurrence and the overall survival time after surgery also approached significance. These findings can be explained by the observations that HAVcR-1 overexpression can reduce colorectal cancer cell adhesion and colorectal cancer cell invasion. As migration of tumour cells is a crucial factor in the development of direct invasion of a tumour this links the expression of HAVcR-1 to the possible control of invasion of colorectal cells.

The involvement of Rho GTPases in major aspects of cancer development, such as cell proliferation, apoptosis, cell polarity, adhesion, migration, and invasion (30) have recently been attracting increasing attention. The Rho GTPase members Ras homolog gene family member A (RhoA), Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division control protein 42 homolog (Cdc42) are involved in the development of colorectal cancer and act as modulators of colorectal cancer development *via* events associated with cancer progression, such as loss of cell-cell adhesion, proliferation, migration, and invasion (30). Our previous study in endothelial cells showed that HAVcR-1 interacts with RhoC (7); this offers a tantalizing glimpse into a possible mechanism for the control of colorectal cancer progression and requires future investigation. Moreover, colorectal tumours can increase their ability to develop haematogenous metastases by inducing the development of new vessels in and around the edges of the tumour. The degree of new vessel formation around the tumours edge was found to be the most important prognostic factor for the development of haematogenous metastases (31). The differential expression of HAVcR-1 in both colorectal tumours and endothelial cells and its role in invasion and TJ formation could provide a useful tool for further investigation.

To our knowledge, there are no other studies investigating the possible expression and role of HAVcR-1 in human colorectal cancer. Cancer studies have concentrated on renal cell carcinoma. However, its use as a biomarker of sufficient sensitivity and specificity for metastatic clear cell renal cell carcinoma is still under consideration (32). Previous work has concentrated on the role of HAVcR-1 as a receptor in hepatocytes. Interaction between the hepatitis A virus and its receptor has been shown to inhibit Treg-cell function, resulting in an immune imbalance that allows viral expansion with limited hepatocellular damage during early stages of infection- a characteristic of hepatitius A virus pathogenesis (33). It is reasonable to assume that HAVcR-1 has functions apart from being a virus receptor and that it could become a key component in the regulation of colorectal cancer progression.

In conclusion, the present study indicates that HAVcR-1 overexpression may be a diagnostic biomarker for colorectal cancer and a prognostic marker for longer disease-free interval after surgery for colorectal cancer. The good prognosis related to HAVcR-1 overexpression may be attributed to the reduction of adhesion and invasion in three colorectal cancer cell lines when HAVcR-1 was overexpressed in these cells.

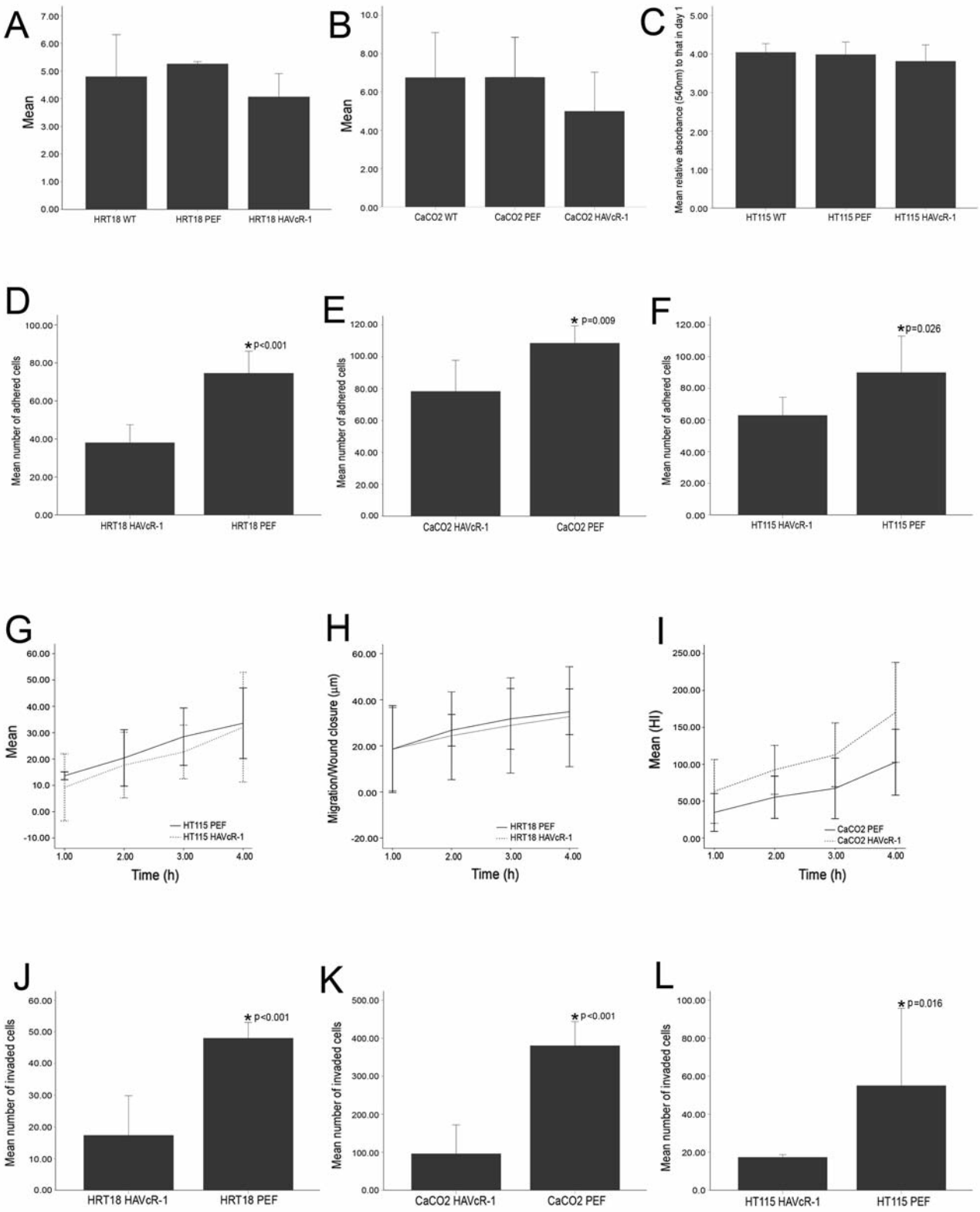


Figure 3. *Cellular function tests of hepatocyte A virus cellular receptor 1 (HAVcR-1) in colorectal cancer cell lines. In vitro cell growth assay for HRT18 (A), CaCO2 (B) and HT115 (C) cells. In vitro cell matrix adhesion assay for HRT18 (D), CaCO2 (E) and HT115 (F) cells. In vitro migration assay for HRT18 (G), CaCO2 (H) and HT115 (I) cells. In vitro invasion assay for HRT18 (J), CaCO2 (K) and HT115 (L) cells.*

## Acknowledgements

We would like to thank Cancer Research Wales and the Albert Hung Foundation for sponsoring this work.

## References

1 Bray F, Ren JS, Masuyer E and Ferlay J: Estimates of global cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer. 2012. Jul 3. doi: 10.1002/ijc.27711.
2 Kim YS andDeng G: Epigenetic changes (aberrant DNA methylation) in colorectal neoplasia. Gut Liver *1*: 1-11, 2007.
3 Feigelstock D, Thompson P, Mattoo P, Zhang Y and Kaplan GG: The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor. J Virol *72*: 6621-6628, 1998.
4 Feigelstock D, Thompson P, Mattoo P and Kaplan GG: Polymorphisms of the hepatitis A virus cellular receptor 1 in African green monkey kidney cells result in antigenic variants that do not react with protective monoclonal antibody 190/4. J Virol *72*: 6218-6222, 1998.
5 Ichimura T, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL and Sanicola M: Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem *273*: 4135-4142, 1998.
6 Kuchroo VK, Umetsu DT, DeKruyff RH and Freeman GJ: The TIM gene family: Emerging roles in immunity and disease. Nat Rev Immunol *3*: 454-462, 2003.
7 Martin TA, Harrison GM, Mason MD and Jiang WG: HAVcR-1 reduces the integrity of human endothelial tight junctions. Anticancer Res *31*: 467-473, 2011.
8 Martin TA, Mason MD and Jiang WG: Tight junctions in cancer metastasis. Front Biosci *16*: 898-936, 2011.
9 Vilà MR, Kaplan GG, Feigelstock D, Nadal M, Morote J, Porta R, Bellmunt J and Meseguer A: Hepatitis A virus receptor blocks cell differentiation and is overexpressed in clear cell renal cell carcinoma. Kidney Int *65*: 1761-1773, 2004.
10 Han WK, Alinani A, Wu CL, Michaelson D, Loda M, McGovern FJ, Thadhani R and Bonventre JV: Human kidney injury molecule-1 is a tissue and urinary tumour marker of renal cell carcinoma. J Am Soc Nephrol *16(4)*: 1126-1134, 2005.
11 Lin F, Zhang PL, Yang XJ, Shi J, Blasick T, Han WK, Wang HL, Shen SS, Teh BT and Bonventre JV: Human kidney injury molecule-1 (hKIM-1): a useful immunohistochemical marker for diagnosing renal cell carcinoma and ovarian clear cell carcinoma. Am J Surg Pathol *31(3)*: 371-381, 2007.
12 Jiang WG, Douglas-Jones A and Mansel RE: Level of expression of lipoxygenase-5, 12, and 15 and cyclooxygenase in human breast cancer. Prostaglaindins Leukotr Ess Fatty Acids *69*: 275-281, 2003.
13 Jiang WG, Davies G, Martin TA, Parr C, Watkins G, Mason MD, Mokbel K and Mansel RE: Targeting matrilysin and its impact on tumour growth *in vivo*: The potential implications in breast cancer therapy. Clin Cancer Res *11*: 6012-6019, 2005.
14 García-Lozano JR, Abad C, Escalera A, Torres B, Fernández O, García A, Sánchez-Román J, Sabio JM, Ortego-Centeno N, Raya-Alvarez E, Núñez-Roldán A, Martín J and González-Escribano MF: Identification of HAVcR-1 gene haplotypes associated with mRNA expression levels and susceptibility to autoimmune diseases. Hum Genet *128(2)*: 221-229, 2010.
15 Chae SC, Song JH, Heo JC, Lee YC, Kim JW and Chung HT: Molecular variations in the promoter and coding regions of human Tim-1 gene and their association in Koreans with asthma. Hum Immunol *64(12)*: 1177-1182, 2003.
16 Chae SC, Song JH, Lee YC, Kim JW and Chung HT: The association of the exon 4 variations of Tim-1 gene with allergic diseases in a Korean population. Biochem Biophys Res Commun *312(2)*: 346-350, 2003.
17 Wang Y, Meng J, Wang X, Liu S, Shu Q, Gao L, Ju Y, Zhang L, Sun W and Ma C: Expression of human TIM-1 and TIM-3 on lymphocytes from systemic lupus erythematosus patients. Scand J Immunol *67(1)*: 63-70, 2008.
18 Xu JR, Yang Y, Liu XM, Sun JY and Wang YJ: Polymorphisms of the TIM-1 gene are associated with rheumatoid arthritis in the Chinese Hui minority ethnic population. Genet Mol Res *11(1)*: 61-69, 2012.
19 Vaidya VS, Ramirez V, Ichimura T, Bobadilla NA and Bonventre JV: Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. Am J Physiol Renal Physiol *290(2)*: F517-F529, 2006.
20 Vaidya VS, Ozer JS, Dieterle F, Collings FB, Ramirez V, Troth S, Muniappa N, Thudium D, Gerhold D, Holder DJ, Bobadilla NA, Marrer E, Perentes E, Cordier A, Vonderscher J, Maurer G, Goering PL, Sistare FD and Bonventre JV: Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol *28(5)*: 478-485, 2010.
21 Page NS, Jones G and Stewart GJ: Genetic association studies between the T-cell immunoglobulin mucin (TIM) gene locus and childhood atopic dermatitis. Int Arch Allergy Immunol *141(4)*: 331-336, 2006.
22 Nuchnoi P, Ohashi J, Kimura R, Hananantachai H, Naka I, Krudsood S, Looareesuwan S, Tokunaga K and Patarapotikul J: Significant association between *TIM1* promoter polymorphisms and protection against cerebral malaria in Thailand. Ann Hum Genet *72(Pt 3)*: 327-336, 2008.
23 Mosbruger TL, Duggal P, Goedert JJ, Kirk GD, Hoots WK, Tobler LH, Busch M, Peters MG, Rosen HR, Thomas DL and Thio CL: Large-scale candidate gene analysis of spontaneous clearance of hepatitis C virus. J Infect Dis *201(9)*: 1371-1380, 2010.
24 Wichukchinda N, Nakajima T, Saipradit N, Nakayama EE, Ohtani H, Rojanawiwat A, Pathipvanich P, Ariyoshi K, Sawanpanyalert P, Shioda T and Kimura A: TIM1 haplotype may control the disease progression to AIDS in a HIV-1-infected female cohort in Thailand. AIDS *24(11)*: 1625-1631, 2010.
25 Kim HY, Eyheramonho MB, Pichavant M, Gonzalez Cambaceres C, Matangkasombut P, Cervio G, Kuperman S, Moreiro R, Konduru K, Manangeeswaran M, Freeman GJ, Kaplan GG, DeKruyff RH, Umetsu DT and Rosenzweig SD: A polymorphism in *TIM1* is associated with susceptibility to severe hepatitis A virus infection in humans. J Clin Invest *121(3)*: 1111-1118, 2011.
26 Kondratowicz AS, Lennemann NJ, Sinn PL, Davey RA, Hunt CL, Moller-Tank S, Meyerholz DK, Rennert P, Mullins RF, Brindley M, Sandersfeld LM, Quinn K, Weller M, McCray PB Jr., Chiorini J and Maury W: T-Cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. Proc Natl Acad Sci USA *108(20)*: 8426-8431, 2011.

27 Hu S, Xie Y, Zhou N, Jin L, Tan Y, Liu D, Gong Y, Liu L, Liu J, Liu W, Chen Y, Zhang Y and Lv N: Expression of T-cell immunoglobulin- and mucin-domain-containing molecules-1 and -3 (Tim-1 and Tim-3) in *Helicobacter pylori* infection. Helicobacter *16(5)*: 373-381, 2011.

28 Zhang Y, Hoffmeister M, Weck MN, Chang-Claude J and Brenner H: *Helicobacter pylori* infection and colorectal cancer risk: Evidence from a large population-based case-control study in Germany. Am J Epidemiol *175(5)*: 441-450, 2012.

29 Ford RM, McMahon MM and Wehbi MA: HIV/AIDS and colorectal cancer: A review in the era of antiretrovirals. Gastroenterol Hepatol (NY) *4(4)*: 274-278, 2008.

30 Leve F and Morgado-Díaz JA: Rho GTPase signaling in the development of colorectal cancer. J Cell Biochem *113(8)*: 2549-2525, 2012.

31 Tanigawa N, Lu C, Mitsui T and Miura S: Quantitation of sinusoid-like vessels in hepatocellular carcinoma: Its clinical and prognostic significance. Hepatology *26(5)*: 1216-1213, 1997.

32 Sangoi AR, Karamchandani J, Kim J, Pai RK and McKenney JK: The use of immunohistochemistry in the diagnosis of metastatic clear cell renal cell carcinoma: A review of PAX-8, PAX-2, hKIM-1, RCCma, and CD10. Adv Anat Pathol *17(6)*: 377-393, 2012.

33 Manangeeswaran M, Jacques J, Tami C, Konduru K, Amharref N, Perrella O, Casasnovas JM, Umetsu DT, Dekruyff RH, Freeman GJ, Perrella A and Kaplan GG: Binding of hepatitis A virus to its cellular receptor 1 inhibits T-regulatory cell functions in humans. Gastroenterology *142(7)*: 1516-1525, 2012.

*Received October 29, 2012*
*Revised November 14, 2012*
*Accepted November 15, 2012*