MicroRNA-214 suppresses growth, migration and invasion through a novel target, high mobility group AT-hook 1, in human cervical and colorectal cancer cells

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Background: MicroRNA-214 (miR-214) has been shown to act as a tumour suppressor in human cervical and colorectal cancer cells. The aim of this study was to experimentally validate high mobility group AT-hook 1 as a novel target for miR-214-mediated suppression of growth and motility.

Methods: HMGA1 and miR-214 expression levels were estimated in cervical and colorectal clinical specimens using qPCR. HMGA1 3’ untranslated region luciferase assays were performed to validate HMGA1 as a target of miR-214. Effect of altering the expression of miR-214 or HMGA1 on proliferation, migration and invasion of human cervical and colorectal cancer cells was investigated.

Results: miR-214 expression was poor while that of HMGA1 was high in cervical and colorectal cancer tissues. miR-214-re-expression or HMGA1 downregulation inhibited proliferation, migration and invasion of cancer cells while miR-214 inhibition had opposite effects. miR-214 was demonstrated to bind to the wild-type 3’ untranslated region of HMGA1 but not with its mutant.

Conclusions: Low expression of miR-214 concurrent with elevated levels of HMGA1 may contribute to cervical and colorectal cancer progression. miR-214-mediated regulation of HMGA1 is a novel mechanism for its tumour-suppressive actions in human cervical and colorectal cancer cells and opens up avenues for novel therapeutic strategies for these two cancers.

Cervical cancer (CaCx) is common among women predominantly in the developing countries (Hawes and Kiviat, 2002), and in India, it is the most common cancer affecting women (Sankaranarayanan et al, 1996). Human papilloma virus (HPV) is the major risk factor for cervical cancer (Bouallaga et al, 2000), but independently, alterations in tumour-suppressor genes and/or oncogenes may also be necessary for cervical cancer progression (Zur Hausen, 1996). Although colorectal cancer (CRC) earlier had the lowest rates of incidence in Asian countries (Haggar and Boushey, 2009), in the past few years, Asia has witnessed a rapid increase in CRC incidence particularly in the developing countries, including India (Mohandas, 2011; Moghimi-Dehkordi, 2012). MicroRNAs are ~22 nucleotide long, small RNA molecules that modulate gene expression transcriptionally or posttranscriptionally by mainly binding to the 3’ untranslated region (UTR) of their target genes (Calin et al, 2002). miRNAs constitute unique expression signatures and modulate cellular signalling pathways in many cancers, including CaCx (Hu et al, 2010; Pereira et al, 2010) and CRC (Chen et al, 2015; Hur et al, 2015). miR-214 functions as a tumour suppressor by downregulating oncogenes, such as GALNT7, Bcl2l2 and TFAM in CaCx (Yang et al, 2009; Peng et al, 2012; Wang et al, 2013; Wen et al, 2014) and FGFR1 and ARL2 in CRC (Chen et al, 2014; Long et al, 2015), but the actual underlying mechanisms are yet to be elucidated. The high mobility group AT-hook (HMGA) proteins are non-histone chromosomal proteins involved in DNA transcription, replication,
recombination and repair (Cleynen and Van de Ven, 2008) and, as recently shown, in the regulation of metabolism (Qiu et al., 2014, 2015). HMGA proteins are easily detected in embryonic, neoplastic and proliferating undifferentiated cells but not so in non-neoplastic human adult tissues (Resar, 2010). They were first discovered in CaCx cells (Lund et al., 1983) and subsequently shown to have an oncogenic role during CaCx initiation, progression and metastasis by cooperating with HPV18 E6/E7 oncoproteins and inactivating p53 (Bandiera et al., 1998; Mellone et al., 2008). Its enhanced expression correlates with tumorigenesis and metastasis in human CRC (Fedele et al., 1996; Huang et al., 2009). Importantly, cis-regulatory elements in the 3′UTR mediate posttranscriptional regulation of HMGA1 (Borrman et al, 2001). Indeed, in leukemia, bladder and prostate cancers, HMGA1 expression is modulated by different miRNAs targeting its 3′UTR (Kaddar et al, 2009; Wei et al, 2011; Lin et al, 2013). In the present study, miR-214 is demonstrated to directly target wild-type 3′UTR of HMGA1 and reduce its endogenous expression in CaCx and CRC cells. Downregulation of HMGA1 expression by miR-214 or anti-HMGA1 significantly inhibits proliferation, migration and invasion of CaCx and CRC cells. Thus novel mechanistic basis for the tumour-suppressive actions of miR-214 is revealed unraveling new therapeutic opportunities.

**MATERIALS AND METHODS**

Human CaCx and CRC tissue samples. Fresh CaCx tissue samples were collected from consenting patients undergoing treatment for CaCx at the Institute of Obstetrics and Gynaecology, Chennai, India. Normal fresh cervical tissues were obtained from patients undergoing hysterectomy for various non-malignant reasons. Fresh CRC tissue samples and adjacent normal tissues were collected from consenting patients undergoing treatment for CRC at the Apollo Hospitals, Chennai, India. The study was approved by the Institutional Ethics Committee of Indian Institute of Technology Madras.

Cell lines. Human cervical cancer cell lines, SiHa, CaSk and C33A, and colorectal cancer cell lines, SW480 and SW620, were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) (Life Technologies, Carlsbad, CA, USA) containing 10% FBS (Life Technologies) and antibiotics (100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin) in a humidified atmosphere of 5% CO₂ at 37 °C.

RNA and plasmid transfection. miRNA-control (no. CN-001000-01-20), miR-214 mimic (no. C-301153-01), siRNA-control (no. D-001220-01-20), siRNA-HMGA1 (no. M-004597-02), anti-miR-control (no. IN-002005-01-20 or anti-miR-214 (no. IH-301153-02-0005) were obtained from GE Healthcare Dharmacon (Lafayette, CO, USA). Transient transfections of the above (with 5 nm of miRNA and anti-miR mimics and 50 nM of siRNA mimics) into CaCx and CRC cells were achieved using Lipofectamine RNAiMAX (no. 13778150, Life Technologies) while pcDNA 3.1, pcDNA 3.1-mir-214, pIRES (vector control), pIRES-HMGA1 (kind gift from Edward Whang, Addgene plasmid no. 13466, Addgene, Cambridge, MA, USA), 3′UTR reporter plasmid constructs were transfected using linear polyethyleneimine (no. 23966-2, MW 25 000, procured from Polysciences, Warrington, PA, USA) at a ratio of 5:1 to DNA. Combinations of miR-control+pIRES, miR-214+pIRES, miR-control+pIRES-HMGA1, miR-214+pIRES-HMGA1 were transfected using DharmaFECT Duo (no. T-2010-01, GE Healthcare Dharmacon).

Western blotting. Total cell lysates were prepared by incubating cells in RIPA lysis buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholate and 1% SDS) on ice for 1 h, and protein concentration was quantified by Bradford’s method according to the manufacturer’s protocol (Bio-Rad, Hercules, CA, USA). Samples (50 µg protein) were resolved on 10% SDS-PAGE and transferred to a PVDF membrane (Immunoblot, Bio-Rad) using a Bio-Rad PROTEAN III apparatus. Anti-HMGA1 (no. 7777S) and anti-ACBT (no. A5441) antibodies were purchased from Cell Signaling (Danvers, MA, USA) and Sigma-Aldrich (St Louis, MO, USA), respectively, while anti-mouse IgG-peroxidase-conjugate (no. 115-035-003) and anti-mouse IgG-peroxidase-conjugate (no. 111-035-003) secondary antibodies were bought from Jackson Laboratories (West Grove, PA, USA). Bands detected using the Enhanced Chemiluminesence Kit (Bio-Rad) were visualised using ChemiDoc (Bio-Rad) and analysed by densitometry (Image Lab, Bio-Rad). ACTB was used as an internal control. Experiments were repeated at least once to confirm the results obtained earlier.

**RNA isolation and real-time quantitative PCR.** Tissue samples were ground and RNA was extracted using the manufacturer’s protocol (TRIzol, Life Technologies) and RNA was also isolated from cells using TRizol (Life Technologies). Mature miRNA levels were estimated by performing stem-loop reverse transcription followed by quantitative PCR; reverse transcription by MMLV reverse transcriptase (Life Technologies) was performed using miR-214-specific and RNU6-specific stem-loop primers. PCR amplification of miR-214 or RNU6 was performed using a forward primer specific for miR-214 or RNU6 (internal control) and a universal reverse primer. For estimating HMGA1 mRNA levels, reverse transcription was carried out by MMLV reverse transcriptase (Life Technologies) using oligo-dt and amplified using appropriate gene-specific PCR primers. Detection and quantitation of HMGA1 or ACTB (internal control) was carried out using the DynaNano ColorFlash SYBR Green qPCR Kit reagent (no. F416L, Thermo Scientific, Waltham, MA, USA) on Eppendorf realplex4 Mastercycler epgradient S (Eppendorf, Hamburg, Germany). Relative expression levels of genes analysed were calculated using 2⁻ΔCt (tissue samples) or 2⁻ΔΔCt (cancer cells) method.

3′UTR luciferase assays. HMGA1 3′UTR which contains putative binding sites for the miR-214 was amplified from human genomic DNA using Phusion high-fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA) and cloned into the 3′UTR of Renilla luciferase gene in the psiCHECK-2 reporter vector (Promega, Madison, WI, USA). The miR-214-binding site was mutated by substituting five out of the six bases in the miRNA-binding sequence (seed sequence) in the 3′UTR of HMGA1 using appropriate primers and the mutant construct thus synthesised was used as a negative control. CaCx or CRC cells were co-transfected with pcDNA3.1 or pcDNA3.1-mir-214 and the wild-type or mutant 3′UTR luciferase constructs in a 24-well format, and 24 h posttransfection, cells were lysed using Passive Lysis Buffer, and Renilla luciferase activity was measured using the Dual Luciferase Assay Kit (no. A2492, Promega) and a luminescence plate reader (Molecular Devices Inc., Sunnyvale, CA, USA), whereby firefly luciferase acted as the internal control.

Migration and invasion assays. Migration assays were performed by transfecting CaCx or CRC cells with miR-214 or siRNA-HMGA1 or anti-miR-214 or respective controls and then seeding 5 x 10⁴ cells in DMEM onto the upper part of each Transwell chamber (BD Biosciences, Franklin Lakes, NJ, USA) and adding 10% FBS containing DEMEM to the lower part of the chamber. Cells adhering to the bottom of the Transwell membrane were stained with 0.1% crystal violet 48 h later and images were obtained using an Olympus TL4 inverted light microscope (Shinjuku, Tokyo, Japan). In addition, stain was collected from stained cells by washing with 10% acetic acid and quantified by measuring absorbance at 595 nm (Saito et al, 1997) using a Bio-Rad Model 680 microplate reader (Bio-Rad, Shinagawa-ku, Tokyo, Japan). Invasion assays were performed in a similar manner but by

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allowing the cells to migrate through a GelTrex-coated (no. A15696-01, Life Technologies) layer in the upper part of the Transwell chamber.

**BrdU incorporation assays.** Proliferation assays were performed by transfecting $0.7 \times 10^7$ cells with miR-214 or anti-miR-214 or siRNA-HMGA1 or respective controls and, 48 h posttransfection, were examined using the BrdU Cell Proliferation Assay Kit (no. 6813S, Cell Signaling) according to the manufacturer’s protocol.

**TCGA data analysis.** TCGA open access data directory (http://cancergenome.nih.gov/) was used to obtain miRNA and mRNA expression data sets for human CaCx and CRC tumours. Normalised TCGA level 3 miRNA-seq and RNA-seqV2 data were compiled using R studio and used for assessing the expression of miRNA and mRNA. These linear sequencing data expression values were then used to compute Pearson product–moment correlation coefficient.

**Statistical analysis.** qPCR was performed in duplicates for all the clinical tissue specimens and mean expression was calculated within normal or cancer tissue subsets. As paired normal specimens were available only in the case of colorectal tumour, miR-214 or HMGA1 expression in a tumour was normalised to its paired normal counterpart. One-way ANOVA test was performed to evaluate fold expression relative to mean. In the case of cervical clinical specimens, mean values for each data set (tumour or normal) were calculated. In the case of miR-control-, miR-214-, siR-control-, si-HMGA1-, anti-miR-control- or anti-miR-214-transfected cells, three independent experiments were conducted for RNA quantitation (in vitro), BrdU incorporation, migration and invasion assays, and after appropriate normalisation, s.e.m. was plotted. After performing unpaired t-tests, P-values were calculated and represented as *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ or ****$P \leq 0.0001$.

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**RESULTS**

**Expression of miR-214 is lower while that of HMGA1 is higher in both human cervical and colorectal tumours than in their corresponding normal tissues.** miR-214 is a known tumour-suppressor miRNA in cervical (CaCx) and colorectal (CRC) cancers and acts by downregulating a few oncogenes as is the case with many tumour-suppressor miRNAs. A putative, conserved, 6-mer, miR-214-binding sequence located at 308–314 bases downstream in the HMGA1 3’UTR was identified using TargetScan. HMGA1 was of particular interest among the target genes identified because of its positive roles in cancer cell proliferation and invasion in CaCx and CRC cells. Prompted by the inverse correlation in the expression of this miRNA–target pair deduced from previous reports that studied miR-214 or HMGA1 expression separately (Fedele et al., 1996; Bandiera et al., 1998; Yang et al., 2009; Chen et al., 2014), these two were analysed together by TCGA data analysis or quantitative PCR in patient samples to strengthen the possibility of the existence of a regulatory mechanism. TCGA analysis showed a clear inverse correlation between miR-214 and HMGA1 both in CaCx and CRC (Figures 1A and B). Pearson correlation coefficients of $-0.239$ ($P = 0.0019$) and $-0.274$ ($P = 0.0005$) were obtained for CaCx and CRC tumour samples, respectively. When a few samples of CaCx and CRC were analysed to check if this correlation existed in a local population, the same trend was found. Expression levels of mature miR-214 analysed in various samples fell into a lower range in CaCx tissues compared with normal tissues (Figure 1C), with mean values of 20.26 and 66.13, respectively. HMGA1 mRNA levels were found in a relatively higher, narrow range (Figure 1D) in CaCx relative to normal tissues with mean values of 22.6 and 8.2, respectively. Similarly, miR-214 was found to be poorly expressed (mean 0.68 ± 0.3), whereas HMGA1 expression was higher (mean 11.52 ± 5.4) in CRC tissues than in their paired normal tissues (Figures 1E and F). Expression levels of miR-214 were higher in 4 out of the 20 CRC samples, whereas HMGA1 expression was lower in 1 out of the 20 tumours when compared with their paired normal tissues. These results showing an inverse relationship between the expression levels of miR-214 and HMGA1 in human CaCx and CRC together with the identification of a miR-214-binding site in the HMGA1 3’UTR suggested that miR-214 may directly target and regulate HMGA1.

**HMGA1 is directly targeted by miR-214.** As the physiological effects of endogenous miR-214 are difficult to ascertain owing to their low levels in CaCx and CRC cells, the miRNA was first re-expressed ectopically. CaCx and CRC cells transfected with miR-214 mimic showed 3–6 and 2–5 fold more expression, respectively, than their corresponding miR-control-transfected cells (Figure 2A). Maintaining this range of expression, the effect of reintroducing miR-214 on endogenous HMGA1 mRNA and protein levels was determined, and the results show that miR-214 decreased HMGA1 both at mRNA (Figure 2B) and protein (Figure 2D) levels in human CaCx and CRC cells. To confirm whether HMGA1 is targeted by miR-214 by binding to its 3’UTR (Figure 2C), HMGA1 3’UTR luciferase assays were performed and it was observed that re-expression of miR-214 decreased wild-type HMGA1 3’UTR-regulated luciferase activity by ~30% in C33A, ~46% in CaSki, ~30% in SiHa, ~25% in SW480 and ~33% in SW620 cells but not in cells transfected with HMGA1 3’UTR-containing mutant miR-214-binding sites (Figure 2E), confirming that miR-214 binds specifically to the 3’UTR of HMGA1 to repress gene expression. Together, these results suggest that miR-214 negatively regulates endogenous HMGA1 expression by binding to its 3’UTR in CaCx and CRC cells.

**Re-expression of miR-214 inhibits proliferation, migration and invasion in CaCx and CRC cells.** To evaluate whether miR-214-mediated targeting and the consequent downregulation/repression of HMGA1 has a role in tumorigenesis, first the effect of ectopic expression of miR-214 on cell proliferation was studied using BrdU incorporation assay in CaCx (C33A and SiHa) and CRC (SW480 and SW620) cells. Re-expression of miR-214 inhibited cell proliferation significantly in C33A by ~28%, SiHa by ~13%, SW480 by ~23% and SW620 by ~18% (Figure 3A). As the CaCx and CRC cells used in the current study are known to exhibit migratory and invasive properties, these cells were next tested by re-expressing miR-214 and it inhibited migration in C33A by ~31%, SiHa by ~23% and SW480 by ~25% (Figure 3B) as well as invasion in C33A by ~25%, SiHa by ~25% and SW480 by ~33% (Figure 3C). These results confirm that reintroduction of miR-214 suppresses the aggressive behaviour of CaCx and CRC cells by inhibiting their proliferation, migration and invasion.

**HMGA1 knockdown inhibits proliferation, migration and invasion in CaCx and CRC cells.** Given that miR-214 may have many potential targets in CaCx and CRC cells, the antiproliferative effect of miR-214 may not be limited to repression of HMGA1. To check whether suppression of HMGA1 would simulate miR-214-mediated effects, siRNA-mediated knockdown of HMGA1 was performed in CaCx and CRC cells (Figure 4A). Under these conditions, cell proliferation was inhibited by 10–40% (Figure 4B). Similarly, diminished levels of HMGA1 reduced migration (Figure 4C) by 30% as well as invasion (Figure 4D) by 20–50%. From these data, it is inferred that merely downregulating HMGA1 expression can inhibit proliferation, migration and invasion in CaCx and CRC cells, producing phenotypes comparable to miR-214 reintroduction.

**Inhibition of miR-214 enhances proliferation, migration and invasion in CaCx and CRC cells.** Although the ectopic expression of miR-214 effectively inhibited proliferation, migration and invasion in
CaCx and CRC cells, whether the endogenous miR-214 contributes to these phenomena remained to be elucidated. When the endogenous miR-214 was inhibited using an antimiR, there was an increase in HMGA1 levels (Figure 5A) and cell proliferation (Figure 5B), migration (Figure 5C) and invasion (Figure 5D) were also enhanced by 40–60%, 30–45% and 20–60%, respectively. From these data, it is ascertained that downregulating miR-214 enhances endogenous HMGA1 levels concurrently stimulating proliferation, migration and invasion of CaCx.

Figure 1. Relative expression levels of miR-214 and HMGA1 in normal and tumour cervical and colorectal tissues. TCGA database was used to plot Pearson product–moment correlation utilising normalised expression data for miR-214 (reads per million (RPM)) and HMGA1 (reads per kilobase per million mapped reads (RPKM)) from (A) 215 cervical and (B) 322 colorectal tumour samples. Quantitative PCR was performed to estimate the expression levels of (C) miR-214 from 20 normal cervical tissue specimens (NT) and 20 cervical tumour tissue specimens (CT) or (D) HMGA1 from 15 normal (NT) and tumour (CT) cervical tissue specimens. Expression levels of miR-214 and HMGA1, respectively, were normalised to that of RNU6 and ACTB. Similarly, 20 tumour (CT) and their paired normal (PN) colorectal tissue specimens were analysed for the expression levels of (E) miR-214 and (F) HMGA1. Solid lines in box plots represent mean values. P-values are represented as *P<0.05, ****P<0.0001.
and CRC cells, producing cellular phenotypes that are considerably opposite to the ones produced by reintroduction of miR-214 or independent downregulation of HMGA1. miR-214 counters the effects of ectopically expressed HMGA1 on proliferation, migration and invasion in CaCx and CRC cells. As miR-214 effectively inhibited proliferation, migration and invasion in CaCx and CRC cells, it was pertinent to test the effects of expressing its target HMGA1 in the presence of miR-214. Hence, miR-214 and a 3′ UTR-less HMGA1 (unresponsive to miRNAs) were transfected individually or in combination in CaCx and CRC cells. As expected, miR-214 decreased HMGA1 expression and ectopic expression of HMGA1 increased it over endogenous levels, and notably, their combined ectopic expression still led to a decrease in HMGA1 in SiHa and SW480 cells (Figure 6A). Similar effects on proliferation (Figure 6B), migration (Figure 6C) and invasion (Figure 6D) were observed when this combination was used. Although miR-214 expression alone reduced proliferation by 14–29%, migration by 22–30% and invasion by 15–27%, ectopic expression of HMGA1 enhanced these. Combined expression of HMGA1 and miR-214 reduced cell proliferation, migration and invasion by 11–29%, 15–37% and 20–25%, respectively, compared with cells transfected with miR-control and vector-control. Taken together, these results ascertain that the tumour-suppressive action of miR-214 prevails over the protumorigenic effects of HMGA1 even if it is ectopically expressed over and above the already abundant endogenous levels in CaCx and CRC cells.

**DISCUSSION**

In recent years, miRNAs have been increasingly demonstrated to have crucial roles in gene regulation, cellular signalling, carcinogenesis and in related processes, including metastasis and epithelial-to-mesenchymal transition (Suzuki et al, 2014; Kuninty...
Figure 3. Effect of ectopic expression of miR-214 on proliferation, migration and invasion. C33A, SiHa, SW480 or SW620 cells were transfected with control mimic (miR-control) or miR-214 mimic and subsequently, (A) BrdU incorporation, (B) migration and (C) invasion were quantified by measuring absorbance. Absorbance values obtained with BrdU or 0.1% crystal violet for miR-214-transfected cells were normalised with those obtained for miR-control-transfected cells. Representative images for migration and invasion are displayed above quantitation. P-values are represented as *P<0.05, **P<0.01, ***P<0.001 or ****P<0.0001. A full colour version of this figure is available at the British Journal of Cancer journal online.
et al, 2016). miRNAs function by modulating the expression of several target genes simultaneously and can act as oncomirs by silencing the expression of tumour-suppressor targets and conversely can target oncogenes and behave as tumour-suppressor miRNAs (Esquela-Kerscher and Slack, 2006). Previously, it has been demonstrated that miR-29b downregulates canonical Wnt signalling and consequently inhibits colorectal cancer cell proliferation and anchorage-independent growth (Subramanian et al, 2014), whereas miR-106b enhances migration and invasion in non-small cell lung carcinoma cells by suppressing β-TRCP2 (Savita and Karunagaran, 2013). Present data obtained from human cervical and colorectal clinical specimens and those extracted from TCGA suggest poor expression of miR-214 in these tumours, confirming earlier reports in these cancers. Inhibition of growth, migration and invasion in CaCx and CRC upon miR-214 re-expression are consistent with earlier data (Peng et al, 2012; Chen

Figure 4. Changes in proliferation, migration and invasion assays upon knockdown of HMGA1. (A) CaCx or CRC cells were transfected with control siRNA (siR-control) or siRNA-HMGA1 (si-HMGA1) and the expression of HMGA1 was checked by western blotting. (B) BrdU incorporation, (C) migration and (D) invasion assays were quantified by measuring absorbance. Absorbance values obtained for si-HMGA1-transfected cells were normalised with those obtained for siR-control-transfected cells. Representative images for migration and invasion are displayed above quantitation. P-values are represented as *P<0.05, **P<0.01, ***P<0.001 or ****P<0.0001.
et al., 2014). However, miR-214 is highly expressed in melanoma (Penna et al., 2013), and gastric (Yang et al., 2013) cancers, and in ovarian cancer, it favours cell survival and cisplatin resistance by targeting the phosphate and tensin homolog (Yang et al., 2008), suggesting that it may function as an oncogene as well. These contradictory results are, however, not uncommon with miRNAs as the same miRNA may act as an oncomir in one cancer type and as a tumour suppressor in another cancer. For instance, miR-155 significantly contributes to growth in B-cell lymphoma (Pedersen et al., 2009) but sensitises triple-negative human breast cancer cells

**Figure 5. Effect of anti-miR-214 on proliferation, migration and invasion.** (A) CaCx or CRC cells were transfected with control anti-miR (anti-miR-control) or anti-miR-214 and the expression of HMGA1 was checked by western blotting. (B) BrdU incorporation, (C) migration and (D) invasion assays were quantified by measuring absorbance. Absorbance values obtained for anti-miR-214-transfected cells were normalised with those obtained for anti-miR-control-transfected cells. Representative images for migration and invasion are shown above quantitation. *P*-values are represented as *P<0.05, **P<0.01, ***P<0.001 or ****P<0.0001.
to infrared by targeting RAD51 (Gasparini et al, 2014). Ectopic expression of miR-214 inhibited cell proliferation more effectively in C33A and SW480 than in SiHa and SW620 cells, and these differential and paradoxical effects are presumably due to differences in genetic background, microenvironment and the pattern of target gene expression in each cell type. Furthermore, multiple miRNAs may exhibit cooperative binding to binding sites on a single target gene (Lewis et al, 2003; Krek et al, 2005), facilitating the formation of complex regulatory networks.

Antithetical to the impairing effect of miR-214 re-introduction on the growth, migration and invasion of CaCx and CRC cells, the presence of HMGA1 is documented to aid tumorigenesis and its maintenance, crucial to metastasis (Reeves et al, 2001; Mellone et al, 2008; Belton et al, 2012; Xing et al, 2014). Downregulating HMGA1 using siRNA suppressed cell proliferation, invasion and migration in this study in a manner comparable to re-expression of miR-214, suggesting that miR-214 mainly acts through HMGA1 or alternatively it emphasises a major role for HMGA1 on the regulation of tumour progression. Further, the finding in the current study that miR-214 could target HMGA1 3'UTR and inhibit its expression lends credence to its tumour-suppressive role. Although downregulation of HMGA1 by miR-16 in leukaemia (Kaddar et al, 2009), miR-142-3p in osteosarcoma (Xu et al, 2014), miR-26a in bladder cancer (Lin et al, 2013) and miR-296 in prostate cancer (Wei et al, 2011) led to inhibition of cancer cell growth and motility, present findings are the first to relate similar phenomena in CaCx and CRC cells. HMGA1 upregulates STAT3 expression in leukaemia (Hillion et al, 2008), is induced by TGF-β
signalling in breast carcinoma (Zu et al, 2015), cooperates with NF-
κB (p65 Rel-A) and bolsters HPV E6/E7 expression via a positive
autoregulatory loop in CaCx (Mellone et al, 2008) and sustains
Wnt/β-catenin pathway in CRC (Xing et al, 2014). Although a
hitherto unknown mechanism by which HMGA1 may be regulated
in CaCx and CRC is shown, it remains to be studied whether other
miRNAs predicted to target HMGA1 may be able to regulate it
simultaneously with miR-214 or independent of it. That a potent
oncogene such as HMGA1 is most effectively regulated by miR-214
is demonstrated by the finding that the ectopic expression of a
3’UTR-less HMGA1 did not act as a deterrent to miR-214 in its
inhibitory effects on proliferation, migration and invasion in CaCx
and CRC cells and conclusively delineates the functional relevance
of the antitumorigenic role of miR-214 in these cancers, through
regulation of HMGA1.

Current data indicate that poor expression of miR-214
concomitant with elevated levels of HMGA1 may contribute to
malignant phenotype in CaCx and CRC. Thus the identification of
HMGA1 as a major target gene for miR-214 in CaCx and
CRC unleashes potential molecular mechanisms of tumorigenesis.

Novel and salient findings from this study in cervical and
colorectal cancers are listed below.

(1) a hitherto unknown mechanism for the tumour-suppressive
actions of miR-214.
(2) miRNA-mediated regulation of HMGA1, and
(3) siRNA-mediated downregulation of HMGA1 and its effect on
proliferation, migration and invasion.

CONCLUSIONS

This is the first report elaborating that miR-214, whose attenuated
expression in CaCx and CRC has been associated with poor
prognosis in previous studies, functions as a tumour suppressor by
negatively regulating expression of oncogene HMGA1. Down-
regulation of cellular HMGA1 alone affected cancer cells in a
manner comparable to that of restoration of miR-214 while
inhibition of endogenous miR-214 resulted in elevated HMGA1
levels and a consequent amelioration of growth, migration and
invasion. Further, ectopic expression of miR-214 was able to thwart
the protumorigenic effect of ectopically expressed HMGA1 and
suppress cancer cell’s development and motility.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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