Loss of the coxsackie and adenovirus receptor contributes to gastric cancer progression

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Loss of the coxsackie and adenovirus receptor (CAR) has previously been observed in gastric cancer. The role of CAR in gastric cancer pathology, however, is unclear. We therefore analysed CAR in 196 R0-resected gastric adenocarcinomas and non-cancerous gastric mucosa samples using immunohistochemistry and immunofluorescence. Cox sackie and adenovirus receptor was found at the surface and foveolar epithelium of all non-neoplastic gastric mucosa samples (n = 175), whereas only 56% of gastric cancer specimens showed CAR positivity (P < 0.0001). Loss of CAR correlated significantly with decreased differentiation, increased infiltrative depths, presence of distant metastases, and was also associated with reduced carcinoma-specific survival. To clarify whether CAR impacts the tumorigenetic properties of gastric cancer, we subsequently determined the role of CAR in proliferation, migration, and invasion of gastric cancer cell lines by application of specific CAR siRNA or ectopic expression of a human full-length CAR cDNA. These experiments showed that RNAi-mediated CAR knock down resulted in increased proliferation, migration, and invasion of gastric cancer cell lines, whereas enforced ectopic CAR expression led to opposite effects. We conclude that the association of reduced presence of CAR in more severe disease states, together with our findings in gastric cancer cell lines, suggests that CAR functionally contributes to gastric cancer pathogenesis, showing features of a tumour suppressor. 

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Gastric adenocarcinoma represents the second-leading cause of cancer-related death worldwide (Parkin et al, 2005). The clinical outcome of gastric cancer is critically determined by the local tumour growth as well as the presence of local and distant metastases (Maehara et al, 2002; Hohenberger and Gretschel, 2003). For both, invasion and metastatic spread, an impaired adhesion of cancer cells is considered a crucial prerequisite. Although previously, in particular, the adherens junction protein E-cadherin (Christofori and Semb, 1999) has been studied, investigation of tight junctions (TJs) in gastric cancer has become of interest in recent years. Hereby, a decreased presence of TJ proteins has been described for claudins 4, 18, and 23, ZO-1 as well as occludin, in part correlating with poor cancer differentiation (Kimura et al, 1997; Katoh, 2005; Lee et al, 2005; Sanada et al, 2006). An increased presence of TJ proteins when compared with normal gastric mucosa has been described for claudins 1, 3, 4, 5, and 7, particularly in intestinal-type adenocarcinomas (Johnson et al, 2005; Resnick et al, 2005; Hewitt et al, 2006; Soini et al, 2006; Wu et al, 2006). These observations point to a complex deregulation of TJ proteins in gastric carcinogenesis, being suggestive of a cancer inhibitory role of downregulated TJ proteins. In contrast, upregulation of TJ proteins may account for tumour-promoting functions as suggested previously for claudin 1 in colon cancer (Dhawan et al, 2005). A more detailed understanding of the functional impact of TJs in gastric cancer, however, is still missing.

The coxsackie adenovirus receptor (CAR), a transmembrane glycoprotein, had initially been characterised as viral attachment site on the surface of epithelial cells (Bergelson et al, 1997). Later on it was identified as a component of the TJ complex, an interacting partner for a number of other TJ proteins, and a regulator of TJ formation (Cohen et al, 2001; Sollerbrant et al, 2003; Coyne et al, 2004; Excoffon et al, 2004; Mirza et al, 2005; Raschperger et al, 2006). On the basis of in vitro assays, it has been speculated that loss of CAR weakens intercellular adhesion, increases proliferation, and promotes migration as well as invasion of cancer cells (Okegawa et al, 2000, 2001; Bruning and Runnebaum, 2003, 2004; Huang et al, 2005; Wang et al, 2005). These findings led to the hypothesis of a tumour suppressive role for CAR in human cancers. In line with this hypothesis, reduced
presence of CAR was found in advanced cancers in part associated with loss of tumour differentiation, increased infiltration, and a poor prognosis (Rau en et al, 2002; Sachs et al, 2002; Matsumoto et al, 2005; Korn et al, 2006; Buscarini et al, 2007; Okegawa et al, 2007). In gastric adenocarcinomas, an immunohistochemical analysis of cancer tissues from 30 patients and 11 non-cancerous controls showed reduced CAR staining intensities in gastric cancer (Heideman et al, 2001).

Intrigued by this observation, we hypothesised that CAR is involved in gastric cancer biology. To test our hypothesis, we determined the presence of CAR in a large series of gastric cancer patients and correlated these data with various clinicopathological patient characteristics. Second, we conducted cell culture experiments to evaluate, whether CAR influences the proliferative, migratory, and invasive capabilities of gastric cancer cells.

**MATERIALS AND METHODS**

**Study population and tissues**

All tissues were obtained from patients (n = 196) who had undergone curative gastrectomy (R0 resection) between 1995 and 2005. Two of the patients received neoadjuvant chemotherapy and exclusion of these individuals from survival analysis did not influence the results. Written informed consent for experimental biomarker analysis was obtained from all patients before analysis. Patients age ranged from 25 to 87 years (mean 64.2 ± 11.7 years). Out of these patients, 54 patients were lost during follow-up, 1 died of unknown cause, and 3 patients died of reasons not related to gastric cancer (mean follow-up time 25.3 ± 22.5 months). Staging and diagnosis of gastric adenocarcinomas was assessed according to the WHO classification (Hamilton and Aaltonen, 2000) and the TNM classification set out by the International Union against Cancer (Wittekind et al, 2003). Representative areas of each tissue specimen were chosen for the construction of tissue microarrays. Samples of non-neoplastic mucosa were obtained from regions furthest away from the tumour. In brief, a minimum of six tissue cylinders of 0.6 mm diameter from each tumour-bearing donor block and 12 tissue cylinders (six from antrum and six from corpus mucosa) from corresponding non-cancerous mucosa, constructing recipient blocks of tissue microarrays, each with a mean of 141 tissue cylinders from 8 – 14 patients were punched. An overall mean of 6.1 spots (s.d. 2.7) for each carcinoma from different tumour areas were eligible for analyses. Four μm sections from the tissue microarrays were mounted on Superfrost-plus slides for the subsequent immunofluorescence and immunohistochemical analysis.

**Immunofluorescence and Immunohistochemistry**

Immunofluorescence and immunohistochemical staining were carried out as described previously (Anders et al, 2003b). In brief, tissue sections were deparaffinised, rehydrated, and submitted to antigen retrieval by microwave treatment. Anti-CAR (H-300: sc-15405, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1: 50) and anti-ZO-1 (Zymed, S. San Francisco, CA, USA; 1: 300) antibodies served as primary antibodies. For immunofluorescence staining, a FITC-conjugated anti-rabbit antibody or a Cy3-conjugated anti-mouse antibody were used as secondary antibodies (both from Molecular Probes, Eugene, OR, USA). Multicolour fluorescence microscopy was carried out using a Zeiss Axiosport microscope (Carl Zeiss AG, Jena, Germany). For immunohistochemistry, a biotinylated goat anti-rabbit immunoglobulin (Vector Laboratories, Burlingame, CA, USA; 1: 400) served as secondary antibody, followed by treatment with streptavidin-biotinylated horseradish peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories). Using diaminobenzidine tetrahydrochloride (Sigma-Aldrich, Munich, Germany), sections were developed and counter-stained with haematoxylin. Staining results for CAR were evaluated by estimating the percentage of epithelial cells showing specific immunoreactivity by an expert pathologist (MV), who was blinded for the clinical data. The CAR status was classified as: negative (no immunoreactivity), weak (0–5% positive cells), moderate (5–50% positive cells), or strong (>50% positive cells). Only samples showing moderate or strong immunoreactivity were considered positive (Juttner et al, 2006).

**Cell culture and generation of stably transfected cell lines**

Gastric cancer cell lines AGS, KATO III, MKN28, and MKN45 were cultured as previously described (Juttner et al, 2006). Chinese hamster ovary cells stably transfected with human full-length CAR cDNA (CHO-CAR; a kind gift of Dr J Bergelson, Division of Infectious Diseases, Children’s Hospital of Philadelphia, PA, USA) and parental CHO cells were cultured in Ham’s F12 medium containing 10% fetal calf serum (FCS). Gastric cancer cell line AGS, showing high CAR expression, were chosen for CAR down-regulation. Hereby, a CAR-specific siRNA: CCAGAGUACCAAGUGAAGACdTdT or a control siRNA: CACAAAAAGUACCGCAAGdTdT cloned into the ‘pSuper’ vector system (Oligoengine, Seattle, WA, USA) were stably transfected into AGS cells using Effectene (Qiagen, Hilden, Germany). Following selection with Puromycin (Sigma-Aldrich), CAR downregulation in a pooled cell population was tested by western blotting. MKN28 and MKN45 cells, showing low CAR level, were selected for CAR overexpression. These cell lines were transfected with a construct in which the human full-length CAR cDNA is expressed under control of the CMV promoter in a pcDNA3.1 expression vector (hCARpcDNA3.1; a kind gift of Dr J Bergelson) or pcDNA3.1 expression vector alone (Invitrogen, Karlsruhe, Germany) using Effectene (Qiagen). Following neomycin selection (Invitrogen) CAR expression in a pooled cell population was determined by real time RT-PCR assay and western blotting.

**Quantitative mRNA determination**

Total RNA was isolated using TRIZOL (Invitrogen) and reversely transcribed with Oligo-dT primers and SuperScript II (Invitrogen). cDNA generated from 50 ng of total RNA was used for real-time quantification using gene-specific primers (Anders et al, 2003b) and the Brilliant QPCR kit (Stratagene, Amsterdam, The Netherlands) on a Stratagene MX3000P cycler. Quantification was performed by the comparative ΔCt method normalising Ct values to β-actin. CDNA derived from CHO and CHO-CAR cells were used as negative and positive controls, respectively.

**Western blotting**

Protein lysates were obtained as previously described (Anders et al, 2003a). Subsequently, equal amounts of protein lysates were loaded on reducing Laemmli gels, immunoblotted with specific antibodies against CAR (H-300: sc-15405, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) or β-actin (Sigma-Aldrich), and detected using the ECL system (Amersham Pharmacia, Piscataway, NJ, USA). Protein lysates of CHO and CHO-CAR cells were used as negative and positive controls, respectively.

**Assessment of gastric cancer cell proliferation**

Cells were seeded onto six-well plates (n = 3 x 10^4 cells per well) in DMEM containing 10% FCS. After 48 h, cells were detached using trypsin and counted using a haemocytometer counting chamber (VWR International, Darmstadt, Germany). All experiments were performed in triplicate and repeated at least twice. Statistical calculations for relationships between CAR status and cell
numbers were carried out using Fisher’s exact probability test (GraphPad Prism software, version 4.00; GraphPad Software Inc., San Diego, CA, USA).

Gastric cancer cell migration
Assessment of directed gastric cancer cell migration was performed using an ‘AP48 48 Well Micro Chemotaxis Chamber’ (Neuro Probe, Gaithersburg, MD, USA). Hereby, 50,000 cells per well in 50 μl FCS-free media were seeded onto the upper part of the chamber, whereas its lower compartment was filled with media containing 10% FCS or serum-free media as a control. After 24 h at 37°C, cells migrated through the filter were fixed with 100% methanol and stained using Diff-Quick reagent (Fisher Scientific, Schwerte, Germany). Non-migrated cells at the upper side of the filter were swiped off. All experiments were performed in triplicate and repeated at least twice.

Gastric cancer cell invasion
Cells were seeded onto the top of ‘BioCoat Matrigel Invasion Chambers’ (BD Biosciences, Bedford, MA, USA) containing 8 μm pore size PET membranes covered with matrigel matrix. Medium containing 10% FCS was added to the bottom well of the chambers as a chemoattractant, whereas serum-free medium was used as a control. Following 24 h at 37°C, cells migrated through the filter were fixed with 100% methanol and stained using Diff-Quick reagent (Fisher Scientific, Schwerte, Germany). Non-migrated cells at the upper side of the filter were swiped off. All experiments were performed in triplicate and repeated at least twice.

Statistical analysis
Statistical calculations were performed using GraphPad Prism software (version 4.00; GraphPad Software Inc.). Relationships between CAR immunopositivity and clinicopathological features were evaluated using Fisher’s exact probability test. Survival was determined from the date of surgery to the time of event (recurrence or death) using the Kaplan–Meier method. Statistical significance of differences in cumulative survival curves was evaluated using log-rank tests. Additional parameters besides CAR showing a \( P \)-value < 0.05 in the univariate study were included in multivariate survival analyses using the Cox proportional hazard method (SPSS Software, Chicago, IL, USA).

RESULTS
Distribution and presence of CAR in non-transformed and malignant gastric tissues
In all samples of non-cancerous gastric mucosa, homogenous CAR immunoreactivity was observed by immunohistochemistry. Coxsackie and adenovirus receptor was restricted to epithelial cells and not found in endothelial, lymphoid, or stromal cells. On a subcellular level, CAR was preferentially localised at the plasma membrane, showing a ‘honeycomb’ appearance typical of junctional staining. Additional immunofluorescence staining for CAR and ZO-1 showed a co-localisation of both proteins at these sites. In contrast, only 56% of gastric adenocarcinoma samples showed CAR positivity (\( P < 0.0001 \)). In CAR positive cancers, CAR immunoreactivity was limited to tumour epithelium. On a subcellular level, cytoplasmic immunoreactivity was noted in some areas of the tumour in addition to apical plasma membrane staining (Figure 1).

CAR presence and clinicopathological parameters
To assess whether loss of CAR correlates with clinicopathological features, we analysed the results for CAR immunoreactivity using the Fisher’s exact probability test. Loss of CAR in gastric cancer correlated significantly with decreased differentiation (\( P = 0.0238 \)), increased infiltrative depths (\( P = 0.0349 \)), and presence of distant metastases (\( P = 0.0016 \)). There was no correlation between CAR and tumour types according to Lauren’s classification and local.

Figure 1 Coxsackie and adenovirus receptor (CAR) presence and distribution in non-transformed gastric mucosa and in gastric adenocarcinomas. Co-immunofluorescence staining for CAR (red A, C) and ZO-1 (green B, C) using specific antibodies visualised by phase contrast microscopy. CAR is exclusively detected in epithelial cells, co-localising with ZO-1 at the apical cell surface (arrow in C) (magnification: ×400). Typical results for CAR staining determined by immunohistochemistry are shown in non-transformed gastric mucosa, showing CAR localisation at the plasma membrane (arrow in D), well differentiated gastric adenocarcinoma, showing apical plasma membrane immunoreactivity (arrow) as well as segmental cytoplasmic staining (arstensks) (E), and lack of CAR immunoreactivity in undifferentiated gastric carcinoma (F) (magnification: ×100, Bar: 200 μm).
tumour spread (Table 1). To evaluate a potential correlation of CAR presence with the survival of gastric cancer patients, the Kaplan–Meyer algorithm was applied. These analyses showed that loss of CAR was associated with shortened carcinoma-specific survival \( (P = 0.025) \). Furthermore, a nonsignificant trend towards shorter disease-free survival \( (P = 0.67) \) in CAR negative cases was observed (Figure 2). Moreover, presence of local and distant metastasis showed a significant association with shortened carcinoma-specific survival, whereas other clinicopathological parameters failed to gain a significant correlation in this analysis (Table 2). The inclusion of patients who died of non-gastric cancer-related causes had no substantial effect on these results (data not shown). Subsequent multivariate analysis showed that CAR does not qualify as independent prognostic factor for carcinoma-specific survival in our cohort. In contrast, presence of lymph node and distant metastases were identified as independent predictors of a poor clinical outcome in this analysis (Table 3).

### Table 1 Correlation of CAR immunopositivity with clinicopathological parameters

| Clinicopathological parameters | No. of cases | CAR positive | \( P \)-value |
|--------------------------------|--------------|--------------|--------------|
| ‘Normal’ mucosa                | 175          | 175 (100%)   | <0.0001      |
| Gastric cancer                 | 196          | 109 (55.6%)  |              |
| Cancer type                    |              |              |              |
| Intestinal                     | 117          | 59 (50.4%)   | 0.8634       |
| Diffus                         | 46           | 24 (52.1%)   |              |
| Tumour differentiation         |              |              |              |
| G1/G2                          | 69           | 46 (66.7%)   | 0.0238       |
| G3/G4                          | 127          | 62 (48.8%)   |              |
| Tumour infiltration            |              |              |              |
| T1/T2                          | 127          | 78 (61.4%)   | 0.0349       |
| T3/T4                          | 69           | 31 (44.9%)   |              |
| Lymph node metastasis          |              |              |              |
| N0                              | 57           | 33 (57.9%)   | 0.6383       |
| N1-3                            | 137          | 74 (54.0%)   |              |
| Distant metastasis             |              |              |              |
| Absent                         | 54           | 40 (74.0%)   | 0.0001       |
| Present                        | 15           | 4 (26.7%)    |              |

NOTE: statistically significant correlations are shown in bold face. Results were calculated by Fisher’s exact test. Divergent numbers of tissue samples assessable for calculations were due to limited accessibility of clinical information, for example, presence of distant metastasis.

CAR expression in gastric cancer cell lines

To evaluate the influence of CAR on gastric cancer biology, we first determined CAR mRNA expression in a panel of four permanent gastric carcinoma cell lines by a real time RT-PCR assay. By normalising \( C_T \) values for CAR expression relative to \( \beta \)-actin levels, highest CAR expression was found in AGS cells. In comparison, MKN28 cells showed about two-fold less CAR mRNA expression, whereas in KATO III and MKN45 cells the lowest CAR mRNA values were found (Figure 3A). In contrast, determination of CAR protein expression by western blotting showed a robust signal in AGS cells only. In MKN45 cells a weak signal was detected, whereas no expression was found in MKN28 and KATOIII, potentially due to limitations in sensitivity of the assay (Figure 3B). On the basis of these findings, we chose AGS cells for CAR downregulation, and
MKN28 as well as MKN45 cells for CAR over expression in the subsequently performed in vitro assays. Western blotting confirmed reduced CAR protein expression in AGS cells upon stable transfection of CAR-specific siRNA, whereas ectopic expression of hCARcDNA in MKN28 and MKN45 cells resulted in a marked increase of CAR protein levels (Figure 3C).

Impact of CAR on proliferation, migration, and invasion of gastric carcinoma cells

The potential influence of CAR on gastric tumour biology was investigated in a series of in vitro experiments. Coxsackie and adenovirus receptor inhibition in AGS cells (AGS\textsuperscript{CAR−negative}) yielded significantly higher cell numbers upon 48 h of cultivation in proliferation assays compared with the respective controls (AGS\textsuperscript{Vector−control}) (Figure 4A). To minimise the chance of gaining misleading results due to cell death, these cells were counted following staining with Trypan blue dye (Sigma-Aldrich) without misleading results due to cell death, these cells were counted following staining with Trypan blue dye (Sigma-Aldrich) without finding considerable differences between AGS\textsuperscript{CAR−negative} and AGS\textsuperscript{Vector−control} cells (data not shown). Using an in vitro migration assay, AGS\textsuperscript{CAR−negative} cells were found to show markedly increased migratory properties in comparison with AGS\textsuperscript{Vector−control} cells (Figure 4B). To test whether these cells migrate in a FCS-directed manner, FCS-free medium controls were included for each cell line. Hereby, no migration of either AGS\textsuperscript{CAR−negative} or AGS\textsuperscript{Vector−control} cells was noted (data not shown). The evaluation of cancer cell invasion showed a marked increase of AGS\textsuperscript{CAR−negative} vs AGS\textsuperscript{Vector−control} cells into matrigel (Figure 4C). In contrast, CAR overexpression in MKN45 cells (MKN45\textsuperscript{CAR−positive}) reduced cell proliferation significantly compared with vector-only transfected MKN45 cells (MKN45\textsuperscript{Vector−control}) (Figure 4D). The investigation of MKN28\textsuperscript{CAR−positive} vs MKN28\textsuperscript{Vector−control} cells did not show significantly different cell numbers. When evaluating these cells in a migration assay, MKN28\textsuperscript{CAR−positive} yielded approximately 50% less migrated cells compared with MKN28\textsuperscript{Vector−control} cells (Figure 4E). Furthermore, a ~75% reduced invasion of MKN28\textsuperscript{CAR−positive} cells was found compared with MKN28\textsuperscript{Vector−control} cells (Figure 4F). When testing MKN45\textsuperscript{CAR−positive} and MKN45\textsuperscript{Vector−control} cells in these assays, no cell migration or invasion was noted (data not shown).

DISCUSSION

Here we report for the first time that loss of CAR in gastric adenocarcinomas correlates with reduced tumour differentiation, tumour growth, distant metastases, and reduced survival. In line with these clinical findings, our in vitro data show that CAR influences proliferation, migration, and invasion of gastric carcinoma cells.

Our observations show that loss of CAR is not a uniform feature of gastric cancers but correlates with tumour differentiation. So far, claudin 4 has been the only TJ protein shown to be lost in correlation with poor gastric cancer differentiation (Lee et al., 2005). Our findings are in line with a limited number of reports showing a correlation between loss of CAR in cancers of the bladder, oesophagus, liver, and pancreas, as well as colon cancers metastatic to the liver (Sachs et al., 2002; Matsumoto et al., 2005; Korn et al., 2006). Whether loss of CAR is a consequence of cancer...
Moreover, these data did not reach statistical significance and therefore, need further confirmation. As these authors determined CAR mRNA expression, diverging results maybe also explained by the usage of different techniques.

Our study shows a significant correlation of CAR negativity with reduced carcinoma-specific survival and a trend towards a shorter disease-free survival. These data are in accordance with the pathophysiological concept of an association between a disturbed intercellular adhesion and a poor prognosis in gastric cancer (Yasui et al., 2005). Particularly, the loss of E-cadherin has been linked to an unfavourable clinical outcome in gastric cancer patients (Yonemura et al., 1995, 2000; Guilford, 1999). However, apart from E-cadherin, only few studies investigated the correlation of TJ proteins with gastric cancer prognosis: reduced expression of Claudin 3 has been associated with a poorer prognosis in intestinal-type tumours, yet failing to gain statistical significance in multivariate analysis (Soini et al., 2006). Furthermore, gastric cancer patients showing down-regulation of claudin 18 had a significantly worse survival, compared with patients with robust expression of this protein (Sanada et al., 2006). In contrast, strong expression of claudin 4 significantly correlated with a decreased survival in gastric cancers (Resnick et al., 2005). Interestingly, CAR upregulation was correlated with a poor clinical outcome as well: On the basis of CAR mRNA expression, a significant correlation between increased CAR levels and a poor overall survival in breast cancer patients was found (Martin et al., 2005). However, as discussed above, these authors measured mRNA expression. This may account for the differences compared with this study and findings in bladder cancer, where loss of CAR expression correlated with decreased cancer-specific survival, but not disease progression, in an univariate analysis (Matsumoto et al., 2005).

Given the significant correlation between loss of CAR and advanced disease, as well as reduced survival of gastric cancer patients, it may be speculated that the CAR facilitates an invasion and migration suppressive role in gastric cancer. To clarify whether CAR functionally influences the migratory, and invasive capabilities of gastric cancer cells, we carried out a series of in vitro invasion and migration assays upon either inhibition or upregulation of CAR. Hereby, we found that specific CAR silencing increases invasion and migration of gastric cancer cells, whereas ectopic CAR expression resulted in the opposite effect. These in vitro findings in conjunction with our observations made in cancer tissue specimens suggest that CAR influences gastric cancer cell migration and invasion, hereby contributing to tumour infiltration and dissemination. Again, our data are in line with the limited number of observations made in other tumour entities: Following retrovirally mediated expression of full-length CAR cDNA in a glioma cell line, cultured in a three-dimensional spheroid model, Huang et al observed a reduced cancer cell invasion (Huang et al., 2005). An inhibitory effect of CAR on cancer cell migration has been seen upon CAR upregulation in ovarian and cervical cancer cell lines (Bruning and Runnebaum, 2004). The increased migration and invasion of cancer cells upon loss of CAR is currently believed to be a consequence of an impaired intercellular adhesion, as has been shown in ovarian and bladder cancer cell lines (Okegawa et al., 2001; Bruning and Runnebaum, 2004; Wang et al., 2005). Moreover, it has been suggested that CAR may be involved in processes during reorganisation of the cytoskeleton and thereby impact cell migration and invasion. Hereby, binding of CAR to actin, as been shown previously, may pose a central phenomenon (Huang et al., 2007). However, a more detailed understanding of mechanisms underlying functions of CAR in cancer cell motility in general, and gastric cancer cell migration and invasion in particular is currently still missing.

In conclusion, our findings suggest that CAR facilitates tumour suppressor functions in gastric adenocarcinomas. Hereby, our findings add to the current understanding of TJs in gastric cancer, as they represent, to our best knowledge, the first report correlating the presence of a TJ protein with functional characteristics of gastric cancer cells.
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