Loss of KCNQ1 expression in stage II and stage III colon cancer is a strong prognostic factor for disease recurrence

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Background: Colorectal cancer (CRC) is the third most common cancer worldwide. Accurately identifying stage II CRC patients at risk for recurrence is an unmet clinical need. KCNQ1 was previously identified as a tumour suppressor gene and loss of expression was associated with poor survival in patients with CRC liver metastases. In this study the prognostic value of KCNQ1 in stage II and stage III colon cancer patients was examined.

Methods: KCNQ1 mRNA expression was assessed in 90 stage II colon cancer patients (AMC-AJCCII-90) using microarray gene expression data. Subsequently, KCNQ1 protein expression was evaluated in an independent cohort of 386 stage II and stage III colon cancer patients by immunohistochemistry of tissue microarrays.

Results: Low KCNQ1 mRNA expression in stage II microsatellite stable (MSS) colon cancers was associated with poor disease-free survival (DFS) \((P = 0.025)\). Loss of KCNQ1 protein expression from epithelial cells was strongly associated with poor DFS in stage II MSS \((P < 0.0001)\), stage III MSS \((P = 0.0001)\) and stage III microsatellite stable colon cancers \((P = 0.041)\). KCNQ1 seemed an independent prognostic value in addition to other high-risk parameters like angio-invasion, nodal stage and microsatellite instability-status.

Conclusions: We conclude that KCNQ1 is a promising biomarker for prediction of disease recurrence and may aid stratification of patients with stage II MSS colon cancer for adjuvant chemotherapy.

With over 1.3 million new patients a year and an estimated 694,000 deaths in 2012, colorectal cancer (CRC) is an increasing healthcare issue, especially in more developed countries (Ferlay et al., 2015). Current guidelines for treatment of primary non-metastatic colon cancer prescribe surgery and adjuvant systemic therapy, the latter based on pathological staging and clinical features. There is consensus that adjuvant chemotherapy generally improves survival in stage III CRC patients without significant comorbidities...
(Moertel et al, 1990; Andre et al, 2009; Haller et al, 2011). For stage II CRC patients this is less obvious and only patients with high-risk features may benefit from adjuvant treatment (Benson et al, 2004; Figueredo et al, 2008; Yother et al, 2011). However, the definition of these high-risk features remains subject of debate. The high-risk clinicopathological features as defined by ASCO-guidelines are perforation or obstruction at presentation, T4, no or poorly differentiated, vascular invasion or inadequate amount of harvested lymph nodes (Benson et al, 2004). With 5-year survival rates ranging from 72 to 83% for stage II and 44–83% for stage III CRC patients, ~25% of stage II CRC patients who do not receive chemotherapy will have disease recurrence. Furthermore a substantial proportion of stage III patients suffers from the side effects of chemotherapy although they might not develop recurrences even if they would not receive adjuvant therapy (O’Connell et al, 2004). Therefore there is a clear clinical need for better prognostic markers to more accurately identify stage II patients at high-risk and stage III patients at low risk of disease recurrence.

Clinical disease course is driven by tumour biology and biomarkers therefore have the potential to complement and improve prognostic value of current clinicopathological features. One example of such a biomarker is microsatellite instability (MSI), which is associated with a more favourable prognosis in stages I and II disease compared with microsatellite stable (MSS) tumours (Sinicropo et al, 2006; Vilar and Gruber, 2010; Sinicropo and Sargent, 2012; de Cuba et al, 2015). Within the group of MSS patients, which comprises 80–85% of all CRCs, there are still major differences in survival, so further stratification is needed for adequate selection of patients who may benefit from adjuvant chemotherapy. Recently, several studies succeeded to classify CRC patients into subgroups with relatively good or poor prognosis, in particular based on RNA signatures (Schlicker et al, 2012; Budinska et al, 2013; De Sousa E Melo et al, 2013; Marisa et al, 2013; Sadanandam et al, 2013; Roepman et al, 2014; Guinney et al, 2015). However, clinical implementation of such gene signatures faces practical hurdles because collection and processing of fresh-frozen tumour samples for RNA isolation is not an established routine workflow in most hospitals, and is logistically demanding. One solution to this problem is to identify prognostic biomarkers that can be implemented in existing routine clinical workflows, such as protein biomarkers that can be evaluated by immunohistochemical staining of formalin-fixed paraffin-embedded (FFPE) tissue material.

We previously identified KCNQ1 as a tumour suppressor gene in mouse and human gastrointestinal cancers (Than et al, 2014). The KCNQ1 gene is located on chromosome 11p15.5 and encodes the β-subunit of voltage-gated potassium channels. KCNQ1 has mainly been studied for its function in the basolateral cell membrane and its predominant presence in cardiac tissue (Peroz et al, 2008), but recently its role in tumourigenesis has gained interest (Starr et al, 2009; March et al, 2011; Than et al, 2014). It was postulated that KCNQ1 mutations have a role in the development of metaplasia, dysplasia and pre-malignant adenomatous hyperplasia and gastric cancer (Elso et al, 2004). In CRC, *Kcnq1* ranked among the highest common insertion site genes in several sleeping beauty DNA-transposon-based forward genetic screens, indicating its putative role as a cancer-driver gene (Starr et al, 2009; March et al, 2011; Takeda et al, 2015). This was further confirmed by studies of *Kcnq1* knockout mice, which exhibited enhanced intestinal tumour multiplicity and tumour progression. Moreover, loss of *Kcnq1* expression in human CRC liver metastases was associated with poor prognosis (Than et al, 2014). Interestingly, β-catenin has been described to regulate KCNQ1 protein expression in cardiac and gastric tissue (Rotte et al, 2009; Wilmes et al, 2012). Indeed, activation of the Wnt signalling pathway in vivo by conditional deletion of APC, in which β-catenin has a crucial role, resulted in increased expression of well-known Wnt-target genes such as *CD44* as well as increased expression of *KCNQ1* (Sansom et al, 2004).

So far, the role of KCNQ1 in colon cancer patients who lack distant metastases has not been established. Therefore, the present study aimed to determine the prognostic value of *KCNQ1* in stages II and III colon cancer patients. We here report that loss of KCNQ1 protein expression is a strong prognostic factor for recurrence and survival in stages II and III colon cancer patients.

**MATERIALS AND METHODS**

**Patient study populations.** The present study made use of two previously published patient series to examine associations of *KCNQ1* to disease-free survival (DFS). The AMC-AJCCII-90 series consisted of 90 stage II colon cancer patients from the Academic Medical Center in Amsterdam, the Netherlands (De Sousa E Melo et al, 2013), and was used to investigate association of *KCNQ1* mRNA expression to DFS. The second series consisted of 226 stage II and 160 stage III colon cancer patients from the Kennemer Gasthuis in Haarlem, the Netherlands (Belt et al, 2011b), and was used to investigate the relation between *KCNQ1* protein expression and DFS. All CRC cases were sporadic except for one patient with Lynch syndrome (HNPCC) and one patient with familial adenomatous polyposis in the AMC-cohort. Both study populations have previously been characterised for MSI status by a five-marker-based PCR analysis system, as described previously (Belt et al, 2012; De Sousa E Melo et al, 2013). Collection, storage and use of clinicopathological data and tissue specimens were performed in compliance with the ‘Code for Proper Secondary Use of Human Tissue in The Netherlands’ (The Code for Proper Secondary Use of Human Tissue (2011)).

**KCNQ1 mRNA expression analysis.** The AMC-AJCCII-90 cohort was previously characterised for genome-wide gene expression by microarray analysis (GSE33113) (De Sousa E Melo et al, 2013). For the present study, *KCNQ1* mRNA expression in tumour tissue was dichotomised in low and high expression using a cutoff that was determined based on optimal separation (R2 platform, http://hgserver1.aml.nl/). Hazard ratios (HR), 95% confidence intervals (95% CI) and *P*-values were calculated using Cox regression analysis. DFS survival curves were visualised by Kaplan–Meier curves and compared using the log-rank test.

**KCNQ1, β-catenin and CD44 protein expression analysis.** The Kennemer Gasthuis series of 386 stages II and III colon cancers was previously used to generate tissue microarrays (TMAs) (Belt et al, 2011b). In brief, three core biopsies (diameter 0.6 mm) were obtained from the centre and three from the periphery of each FFPE tumour tissue donor block and inserted into recipient TMA paraffin blocks using the 3DHISTECH TMA Master (v1.14, 3DHISTECH Ltd, Budapest, Hungary). Protein expression was evaluated by immunohistochemical staining of TMAs.

Sections, 4 μm thick, were mounted on glass slides, deparaffinised by xylene and rehydrated with a decreasing alcohol series. Staining for KCNQ1 was performed following antigen retrieval by microwave heating in citric acid (10 mm, pH 6.0) and endogenous peroxidase neutralisation in 0.3% hydrogen peroxide in methanol for 25 min, as described previously (Than et al, 2014). The primary rabbit polyclonal antibody directed against human KCNQ1 (sc-20816; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was incubated overnight at a 1:200 dilution at 4°C, followed by incubation with anti-rabbit secondary antibodies for 30 min at room temperature (Envision Plus; Dako, Heverlee, Belgium). Secondary antibodies were visualised by liquid diaminobenzidine substrate chromogen system. Slides were counterstained with Mayer’s haematoxylin. FFPE colon tissue was used as positive control. Staining for β-catenin has a crucial role, resulted in increased expression of well-known Wnt-target genes such as CD44 as well as increased expression of KCNQ1 (Sansom et al, 2004).

**Loss of KCNQ1 is a strong prognostic factor in CRC**
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RESULTS

Low KCNQ1 mRNA expression is associated with poor DFS in stage II MSS colon cancer patients. To investigate prognostic value of KCNQ1 mRNA expression in non-metastatic CRC patients, we made use of existing gene expression data from the AMC-AJCCII-90 study population consisting of 90 stage II colon cancer patients (De Sousa E Melo et al, 2013). Overall, no significant association between KCNQ1 mRNA expression levels and DFS was observed (Figure 1A). Because MSI status is a known confounding factor with prognostic value in stage II CRC patients, (Vilar and Gruber, 2010; Sinicrope and Sargent, 2012) the association between KCNQ1 mRNA expression and DFS was analysed in MSS (n = 65) and MSI tumours (n = 25) separately. Low expression of KCNQ1 was associated with poor survival in patients with stage II MSS tumours (HR 3.35, 95% CI 1.16–9.66, P = 0.025; Figure 1B) but not in patients with MSI tumours (HR 0.24; 95% CI 0.03–2.15; P = 0.20; Figure 1C). These mRNA data indicate that KCNQ1 has prognostic potential in non-metastatic CRC.

Figure 1. Association of KCNQ1 mRNA expression with DFS of (A) stage II colon cancer patients (AMC-AJCCII-90 study population); (B) the subset of MSS patients; and (C) the subset of MSI patients. Cox regression HR (95% CI) and log-rank P-values are reported.
loss of KCNQ1 is a strong prognostic factor in stages II and III colon cancer. To analyse the added value of KCNQ1 protein expression to current clinicopathological parameters, a multivariate model for 5-year DFS was built using stepwise backward Cox-regression. The variables ‘isolated tumour deposits’, ‘nodal stage’, ‘angio-invasion’ and ‘MSI status’ were retained in the multivariate model for 5-year DFS. The variables were selected as predictive factors for poor DFS in the entire study population, with added value to established clinicopathological factors (HR 3.75; 95% CI 2.50–5.64; P < 0.0001, Table 2). These data demonstrate that loss of KCNQ1 expression is a strong predictor for poor DFS in the entire study population, with added value to established clinicopathological factors (HR 3.75; 95% CI 2.50–5.64; P < 0.0001, Table 2).

KCNQ1 expression is correlated to expression of β-catenin and CD44. It has been postulated that expression of KCNQ1 is regulated by β-catenin and as such results from activation of the Wnt signalling pathway (Sansom et al, 2004; Rotte et al, 2009; Wilmes et al, 2012). TMA’s were stained for β-catenin and the Wnt-target gene CD44. Dichotomised expression was used to investigate whether KCNQ1 expression was correlated to active Wnt signalling. Indeed, KCNQ1 expression was significantly correlated to expression of β-catenin and CD44 in MSS colon cancer patients who received adjuvant chemotherapy (HR 1.98; 95% CI 1.02–3.84; P = 0.041; Figure 4C). In patients who did not receive chemotherapy, low expression of KCNQ1 was associated with poor DFS in stage II patients (HR 4.06; 95% CI 2.04–8.09; P < 0.0001; Figure 4A) and stage III patients (HR 3.01; 95% CI 1.25–7.22; P = 0.014; Figure 4C). In patients who did receive chemotherapy, low expression of KCNQ1 was associated with poor DFS in stage II patients (HR 2.88; 95% CI 1.44–5.77; P = 0.002; Figure 4D). No association was observed between KCNQ1 expression and stage II patients who received adjuvant therapy (Figure 4B); however, it should be noted that this subgroup of patients was relatively small (n = 24). Collectively, these data indicate that loss of KCNQ1 expression is a strong prognostic biomarker to identify stages II and III colon cancer patients at high-risk for disease recurrence, irrespective of adjuvant chemotherapy.
cancers ($\chi^2$-test $P=0.003$ and $P=0.001$, respectively), but not in MSI colon cancers ($P=0.076$ and $P=0.12$, respectively). Despite these significant correlations, expression of nuclear $\beta$-catenin was not significantly associated with DFS (HR 0.83; 95% CI 0.58–1.21; $P=0.33$; Figure 5A). In contrast, low expression of CD44 was associated with poor DFS (HR 2.77; 95% CI 1.35–5.68; $P=0.005$; Figure 5B). However, the association of CD44 with DFS was less strong than that of KCNQ1, and was lost upon further analyses of subgroups of colon cancer patients based on stage, MSI status or adjuvant treatment.

**DISCUSSION**

The present study addressed the prognostic value of KCNQ1 expression in stages II and III colon cancer patients. We here demonstrate that loss of expression of KCNQ1 in epithelial cells is strongly associated with a high risk for disease recurrence, and has additional prognostic value to clinicopathological features that are currently used to select high-risk stage II patients such as angio-invasion, nodal stage and MSI status. These findings are in line with our previous observation that KCNQ1 expression in CRC liver metastases was associated with poor overall survival (Than et al, 2014). Importantly, the prognostic value of KCNQ1 was clearly apparent in both stages II and III MSS colon cancers. In stage III tumours, its prognostic value was evident irrespective of treatment with adjuvant chemotherapy. Moreover, the prognostic value of KCNQ1 was also demonstrated in the smaller subgroup stage III MSI cancers. Only for stage II MSI tumours no significant association was observed between KCNQ1 and DFS, probably due to the limited number of patients in this subgroup combined with the fact that these patients have a relatively good prognosis (Vilar and Gruber, 2010; Sinicrope and Sargent, 2012). Taken together, these data show that loss of KCNQ1 expression from neoplastic epithelial cells is a biomarker that strongly predicts disease recurrence and poor survival in stages II and III colon cancer.

At present, the most promising prognostic biomarkers for stage II colon cancer are based on mRNA signatures (Schlicker et al, 2012; Budinska et al, 2013; De Sousa E Melo et al, 2013; Marisa et al, 2013; Sadanandam et al, 2013; Roepman et al, 2014; Guinney et al, 2015). Making use of the external AMC-AJCCII-90 cohort from which one of these mRNA signatures was derived (De Sousa E Melo et al, 2013), we showed that low KCNQ1 mRNA levels were associated with poor prognosis of stage II MSS patients. Protein expression of KCNQ1 was analysed in a second and independent cohort, which confirmed its prognostic value and demonstrated an even stronger association with DFS. Differences between KCNQ1 mRNA and protein analysis may be due to the fact that mRNA levels are determined on tissue lysates, which include cells from both neoplastic epithelial origin as well as non-neoplastic cells in the tumour stromal compartments. In contrast, KCNQ1 protein analysis was focused on expression in neoplastic epithelial cells only, and its score is therefore not affected by variation in tumour stromal content. The hazard rate ratio for KCNQ1 protein expression in the present study (HR > 3.5 for stage II and HR > 4.0 for stage II MSS patients) was higher than that of biomarkers that have been identified previously using this study population, such as lamin A/C, AURKA, versican, lumican, Bcl-XL, FAS, Bcl-2 and FasL (Belt et al, 2011a; Belt et al, 2011b; de Wit et al, 2013; Belt et al, 2014). Only MGL ligand has been identified as a biomarker with comparatively strong effects in this study population, albeit restricted to the subgroup of stage III colon cancers (Lenos et al, 2015). Interestingly, the prognostic value of KCNQ1 protein expression in stage II CRC patients in the present study is comparable to or better than the HRs reported for validated gene expression signatures like OncotypeDX Colon Cancer (HR’s ranging from 1.58 to 1.96 (Gray et al, 2011; Venook et al, 2013; Yothers et al, 2013)), ColoPrint (HR’s ranging from 2.16 to 2.65 (Salazar et al, 2011; Maak et al, 2013).
Kopetz et al., 2015), and GeneFx Colon (HR 2.53 (Kennedy et al., 2011)). Moreover, the protein staining-based KCNQ1 HRs exceed the mRNA signature-based HRs for differences between the poor survival CMS4 group and the CMS1–3 groups of colon cancers (Guinney et al., 2015), implying that KCNQ1 may have a stronger prognostic value than the consensus-based mRNA signatures. Although collection of frozen tissue for mRNA isolation is not common clinical practice, protein analysis by immunohistochemistry of FFPE material is. As such, implementation of KCNQ1 as a prognostic biomarker is technically well feasible in the current clinical setting.

Nuclear β-catenin and CD44 expression have commonly been used to indicate ‘stemness’ of colon cancer cells. Because β-catenin has been described to regulate KCNQ1 protein expression (Wilmes et al., 2012) and KCNQ1 expression was shown to be increased upon activation of the Wnt signalling pathway (Sansom et al., 2004), the correlation between KCNQ1 expression and nuclear β-catenin was examined, as well as with the Wnt-target gene CD44. The current data demonstrated a positive correlation of KCNQ1 expression with both genes, and like for KCNQ1 also loss of CD44 expression was associated with poor DFS. In contrast, expression of nuclear β-catenin was not significantly associated with DFS. Therefore, while high Wnt-activity is thought to lead to development and progression of cancer, our data indicate that high expression of KCNQ1 was associated with better prognosis. As such, the present findings lend further support to previous observations in which silencing of several Wnt target genes by methylation-stimulated tumour progression and was associated with poor prognosis (de Sousa E Melo et al., 2011; Silva et al., 2014). This hypothesis is further supported by the observation that loss of expression of KCNQ1 is significantly more prevalent in MSI tumours (54%) than in MSS

Table 1. Clinicopathological characteristics of 377 colon cancer patients, stratified for low and high KCNQ1 protein expression

| Clinicopathological characteristics | Total: n = 377 (%) | KCNQ1-low: n = 136 (%) | KCNQ1-high: n = 241 (%) | P-value |
|------------------------------------|--------------------|------------------------|------------------------|---------|
| **Sex**                            |                    |                        |                        |         |
| Male                               | 197 (52.3)         | 68 (50.0)              | 129 (53.5)             | 0.51    |
| Female                             | 180 (47.7)         | 68 (50)                | 112 (46.5)             |         |
| **Age, median (min–max) in years** |                    |                        |                        |         |
| 72.9 (28.5–94.0)                   | 72.7 (34.7–94.0)   | 73.2 (28.5–92.3)       | 0.80      |
| **Right-sided tumour**             | 170 (45.1)         | 73 (53.7)              | 97 (40.2)              | 0.012   |
| **Tumour diameter, median (min–max) in mm** | 40.0 (10–130) | 40 (12–100)              | 35 (10–130)             | 0.09    |
| **Histological grade**             |                    |                        |                        |         |
| Well differentiated                | 24 (6.4)           | 9 (6.6)                | 15 (6.2)               |         |
| Moderately differentiated          | 296 (78.5)         | 97 (71.3)              | 199 (82.6)             |         |
| Poorly differentiated              | 57 (15.1)          | 30 (22.1)              | 27 (11.2)              | 0.017   |
| **Stage**                          |                    |                        |                        |         |
| II (= N0)                          | 223 (59.2)         | 76 (55.9)              | 147 (61.0)             |         |
| III (= N+)                         | 154 (40.8)         | 60 (44.1)              | 94 (39.0)              | 0.33    |
| **Tumour stage**                   |                    |                        |                        |         |
| T1                                 | 4 (1.1)            | 1 (0.7)                | 3 (1.2)                |         |
| T2                                 | 18 (4.8)           | 5 (3.7)                | 13 (5.4)               |         |
| T3                                 | 318 (84.3)         | 112 (82.4)             | 206 (85.5)             |         |
| T4                                 | 37 (9.8)           | 18 (13.2)              | 19 (7.9)               | 0.34    |
| **Nodal stage (stage III)**        |                    |                        |                        |         |
| N1                                 | 107 (28.4)         | 37 (27.2)              | 70 (29.0)              |         |
| N2                                 | 47 (12.5)          | 23 (16.9)              | 24 (10.0)              | 0.09    |
| **No. of lymph nodes, mean (s.d.)**|                    |                        |                        |         |
| Positive (in stage III), mean (s.d.) | 8.9 (5.2)     | 8.7 (5.0)              | 9.0 (5.3)              | 0.57    |
| - Mucinous differentiation          | 80 (21.2)          | 44 (32.4)              | 36 (14.9)              | <0.001  |
| **MSI-status**                     |                    |                        |                        |         |
| MSI                                | 63 (16.7)          | 34 (25.0)              | 29 (12.0)              | 0.001   |
| MSS                                | 262 (69.5)         | 82 (60.3)              | 180 (74.7)             |         |
| Unknown                            | 52 (13.8)          | 20 (14.7)              | 32 (13.3)              |         |
| **Ulceration**                     | 290 (76.9)         | 104 (76.5)             | 186 (77.2)             | 0.88    |
| **Angio-invasion**                 | 73 (19.4)          | 33 (24.3)              | 40 (16.6)              | 0.07    |
| **Emergency surgery**              | 51 (13.5)          | 23 (16.9)              | 28 (11.6)              | 0.15    |
| **Perforation**                    |                    |                        |                        |         |
| No                                 | 347 (92.0)         | 122 (89.7)             | 225 (93.4)             |         |
| Before surgery                     | 15 (4.0)           | 9 (6.6)                | 6 (2.5)                |         |
| During surgery                     | 5 (1.3)            | 2 (1.5)                | 3 (1.2)                |         |
| After surgery                      | 10 (2.7)           | 3 (2.2)                | 7 (2.9)                | 0.26    |
| **Tumour spill**                   | 12 (3.2)           | 5 (3.7)                | 7 (2.9)                | 0.68    |
| Adjuvant chemo                     | 122 (32.6)         | 46 (33.8)              | 76 (31.5)              | 0.65    |
| Recurrence                         | 123 (32.6)         | 74 (54.4)              | 49 (20.3)              | <0.001  |
| **CRC mortality**                  | 98 (26.0)          | 58 (42.6)              | 40 (16.6)              | <0.001  |
| Overall mortality                  | 173 (45.9)         | 76 (55.9)              | 97 (40.2)              | 0.003   |
| Follow-up, median (min–max) in months | 57.2 (2.8–148.6) | 48.1 (2.8–129.2)     | 61.2 (4.14–148.6)     | <0.001  |

Abbreviation: CRC = colorectal cancer. P-values were calculated by χ²-test or independent t-testing for continuous data. Significant P-values are printed in bold.

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tumours (31%). MSI is frequently caused by silencing of MLH1 in tumours with a CpG island methylator phenotype (Cancer Genome Atlas Network, 2012; Kim et al., 2013). These data suggest that aberrant methylation is at least one mechanism that can cause silencing of KCNQ1 expression, and show that loss of KCNQ1 expression has a prominent role in the metastatic potential of both MSS and MSI cancers. Although KCNQ1 is not included in gene panels that are used to determine the CIMP phenotype in colorectal carcinomas (Weisenberger et al., 2006), DNA hypermethylation of KCNQ1 is considered a hallmark of CIMP in renal cell cancer (Arai et al., 2014).

In the present study, MSI tumours were overrepresented among right-sided tumours, consistent with literature (Benatti et al., 2005; Buecher et al., 2013). Consequently, loss of KCNQ1 protein expression was also associated with right-sided tumours (Table 1). In multivariate analysis, however, tumour location was not retained in the model while expression of KCNQ1 and MSI-status were (Table 2).

Functionally the KCNQ1 gene encodes for a potassium channel protein. Potassium channels have an important role through their ion-channel function, with effects on proliferation, differentiation and apoptosis (Lehen’kyi et al., 2011). The active efflux of potassium changes its cellular concentrations with a subsequent passive shift of chloride regulated by CFTR, the gene affected in patients with cystic fibrosis. Transport of these ions causes the flow of water in and out of cells, thereby regulating cell volume, which is known to have direct effects on apoptotic pathways in normal cells (Pedersen et al., 2013). In this way, the function of ion channels such as KCNQ1 and CFTR can affect cell survival (Than et al., 2014, 2016). Indeed, dysfunctional ion channels and cell volume regulation have been associated with resistance to apoptosis and resistance to chemotherapy (Pedersen et al., 2013). However, the exact role of KCNQ1 amongst all K+ -channel proteins in this context is not known and therefore the relation of KCNQ1 expression to apoptosis and proliferation needs further elucidation. Furthermore, we previously demonstrated that Kcnq1-deficient tissues are enriched in Cfrt-deficient gene signatures as well as in Muc2-deficient signatures (Than et al., 2014). The Muc2 gene encodes for the main component of the protective mucus barrier Mucin2, which has a tumour suppressive role (Yang et al., 2008). Disruption of normal CFTR function can also lead to a diminished mucus barrier that protects intestinal epithelial cells from bacterial contact (Gustafsson et al., 2012). These data suggest that loss of KCNQ1 can lead to excessive activation of innate immune signalling that may induce cancer-promoting inflammation.

In summary, we conclude that KCNQ1 is a strong prognostic biomarker for prediction of disease recurrence in stages II and III colon cancer patients. In particular, this biomarker could be very useful to stratify patients with stage II MSS for adjuvant therapy.

Figure 4. DFS curves for expression of KCNQ1 in MSS colon cancer patients not treated (A and C) and treated (B and D) with adjuvant chemotherapy for stage II (A and B) and stage III (C and D) disease. Cox regression HR (95% CI) and log-rank P-values are reported.

| Parameters                  | HR     | 95%-CI  | P-value |
|-----------------------------|--------|---------|---------|
| Isolated tumour deposits    | 1.594  | 0.98–2.39 | 0.06   |
| Nodal stage                 | 1.683  | 1.30–2.18 | 0.0001 |
| Angio-invasion              | 2.062  | 1.32–3.22 | 0.001  |
| MSI status                  | 0.589  | 0.34–1.03 | 0.06   |
| Low KCNQ1-expression        | 3.752  | 2.50–5.64 | <0.0001|

Abbreviations: CI = confidence interval, HR = hazards ratio, MSI = microsatellite instability.
therapy, since selection of patients who might benefit from such treatment is still challenging. Further studies are needed both for prospective validation in a clinical setting and to address the functional role of KCNQ1 in cell homoeostasis, Wnt signalling and CRC development.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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**Figure 5.** Association of DFS with expression of (A) nuclear β-catenin and (B) CD44. Cox regression HR (95% CI) and log-rank P-values are reported.
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