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Serum macrophage inhibitory cytokine-1 (MIC-1/GDF15): a potential screening tool for the prevention of colon cancer?

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Abstract

[§]Disclosure statement

David A. Brown, Kenneth W. Hance, Connie J. Rogers, Elaine Lanza and Samuel N. Breit are co-inventors on patents filed by St Vincent's Hospital, and the NIH, which pertain to the use of a serum-based assay for MIC-1/GDF15 in colon cancer.

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Author Contribution

DAB, SNB, KWH and EL conceived the study. EL, AS, PSA and AJC conceived the original polyp prevention trial, administered the enrolment of patients, collection and storage of samples and related data as well as curating of the database. DAB and SNB performed MIC-1/GDF15 serum measurement. DAB, KWH, LBS, PSA, ZW, AJC, PS, JA and ML performed statistical analysis and had access to the data set. DAB, KWH, LBS, PSA, GM, AJC ZW, AJC, PS, JA, AOL, ML and EL interpreted the data and participated in manuscript preparation and review.

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Background—Macrophage inhibitory cytokine-1 (MIC-1/GDF15) mediates NSAID protection from colonic polyps in mice and is linked to the development of colorectal carcinoma in humans. Therefore, changes in serum MIC-1/GDF15 levels could predict the presence of pre-malignant colonic polyposis and assist in population screening strategies.

Methods—Serum MIC-1/GDF15 levels were measured in subjects in the Polyp Prevention Trial, where NSAID use and colon cancer risk factors were defined. Subjects had an initial adenoma removed, a repeat colonoscopy removing previously unidentified polyps, and serum MIC-1/GDF15 estimation. Three years later recurrent adenomas were identified and serum MIC-1/GDF15 levels re-estimated. The relationship between serum MIC-1/GDF15 levels and adenoma presence or recurrence was examined.

Results—Serum MIC-1/GDF15 levels differed by adenoma status and were significantly related to colon cancer risk factors. Additionally, mean serum MIC-1/GDF15 levels rose with increasing numbers of adenomas present and high-risk adenoma recurrence. NSAID users had higher serum MIC-1/GDF15 concentrations, which were related to protection from adenoma recurrence. Further, adjusted serum MIC-1/GDF15 levels at final follow up were related to adenoma recurrence (highest quartile MIC-1/GDF15; OR 14.7 95%CI 3.0–73).

Conclusions—These data suggest that MIC-1/GDF15 mediates at least some of the protection afforded by NSAIDs against human colonic polyposis. Further, serum MIC-1/GDF15 levels vary with the development of adnenomatous colonic polyps.

Impact—Serum MIC-1/GDF15 determination may hold promise as the first serum screening test to assist the detection of pre-malignant adenomatous colonic polyposis.

Keywords

MIC-1; GDF15; colonic polyposis; colon cancer; screening and prevention

Introduction

The transforming growth factor-beta (TGF-β) superfamily member Macrophage inhibitory cytokine-1 (MIC-1/GDF15) is present in the serum of all normal individuals with a normal range of 150–1150 pg/ml (1). Elevated serum levels of MIC-1/GDF15 have been reported in patients with many cancers, including colorectal neoplasia (1–7). Serial analysis of gene expression indicated that MIC-1/GDF15 was one of nine secreted or cell surface expressed colonic adenomas / carcinoma protein transcripts highly upregulated, relative to normal colonic epithelium (8). Additionally, MIC-1/GDF15 protein is easily detectible in both colonic adenomas and carcinomas (1). Consistent with these findings, MIC-1/GDF15 serum levels progressively increase with development of colonic adenomas, high-grade dysplasia, localized and then advanced colonic carcinoma (1).

Expression of MIC-1/GDF15, at least in cell lines, is upregulated by p53 (9) and NSAIDs, the latter through the induction of the transcription factor early growth response protein-1 (10). NSAID induced expression of MIC-1/GDF15 has been reported in many cancer cell lines (11–14) and is associated with pro-apoptotic activity *in vitro* and *in vivo* (11, 12, 15). MIC-1/GDF15 gene KO mice, when crossed with adenomatous polyposis coli gene mice

(APC$^{Min/+}$), lose the protection from colonic polyposis development afforded by NSAID treatment (16). Additionally, over expression of human MIC-1/GDF15 in APC$^{Min/+}$ mice suppresses azoxymethane induced colonic tumor formation (17, 18). These findings support a role for MIC-1/GDF15 in suppressing early colonic neoplasia and suggest that MIC-1/GDF15 may partly mediate NSAID chemoprevention of colonic neoplasia. Finally, in a very small exploratory pilot experiment, serum MIC-1/GDF15 levels decreased, and in one case halved, after removal of a colonic adenoma (unpublished data). Therefore, whilst MIC-1/GDF15 may inhibit the development of colonic adenomas, once present, atypical colonic epithelium also produces MIC-1/GDF15. These expression characteristics suggested that serum MIC-1/GDF15 might be a useful tool to predict colonic adenoma presence. However, adjustment might be needed for potential confounding factors such as MIC-1/GDF15 derived from colonic (adenomas) and/or non-colonic sources, as well as induction by NSAID use. We therefore sought to test this hypothesis in the best available cohort.

To date there has been no serum marker of pre-malignant colonic disease. Because of this, prospective cohorts examining this condition have not prioritized serum collection, particularly with respect to the timing of collection before polypectomy. Indeed, many do not collect serum. This severely limits the number of existing cohorts that have appropriate timed blood sampling to test our hypothesis that serum MIC-1/GDF15 levels can predict the presence of colonic adenomas. The best available cohort was the Polyp Prevention Trial (19–21). These prospectively collected data allowed for the examination of single and serial measurements of serum MIC-1/GDF15 concentrations in relation to colonic adenoma, NSAID use and known risk factors for colorectal cancer. Additionally, we undertook an assessment of serial serum MIC-1/GDF15 level determinations for the prediction of adenoma recurrence. Even this “best available” cohort had significant limitations, nonetheless we were able to show that single and serial serum MIC-1/GDF15 levels were associated with the presence of premalignant colonic adenomas. These data justify the significant expense of appropriately designed prospective trials to examine the role of serum MIC-1/GDF15 measurement in the management of pre-malignant colonic polyposis.

Materials and Methods

Study population.

Participants in this study were 35 years or older with at least one histologically confirmed adenoma removed during a qualifying colonoscopy and were randomized to the control arm of the PPT (19–23). Blood samples from the intervention arm were not available for analysis. Eligible participants had no history of colorectal cancer, surgical resection of adenomas, bowel resection, polyposis syndrome, or inflammatory bowel disease. Of a total of 2,079 participants, 1,042 were assigned to the control arm of the trial and 947 completed the study with 626 (66.1%) having serum available from T1 and T4 (1 and 4 years after baseline) for the analysis of MIC-1/GDF15. Three subjects were excluded after diagnosis of cancer during the study, leaving 623 subjects for analysis. Serum MIC-1/GDF15 level was determined in all patients. However, for determining the utility of serial MIC-1/GDF15 serum levels for adenoma detection, the time of blood sampling was inappropriate in a significant number of subjects (n=370, 59%). Inappropriate timing of blood sampling

included sampling MIC-1/GDF15 prior to adenoma removal at T1, or after an adenoma had been removed at T4. Additionally, in some patients NSAID usage, which may affect serum MIC-1/GDF15 levels, changed during the course of the study. Accordingly, we identified two additional patient subsets that we called 1. 'T1-adenoma free' (n=528 (85%)); and 2. 'Adenoma/NSAID appropriate' (253 (41%)). The 'T1-adenoma free' subset was made up of patients that had their serum MIC-1/GDF15 level measured at T1 and had no adenoma at this screening exam, or MIC-1/GDF15 was measured after their adenoma was removed at T1. From this group of patients, the 'Adenoma/NSAID appropriate' subjects were defined as those who had no adenoma recurrence or had their serum MIC-1/GDF15 level measured prior to adenoma removal at T4 and did not change their NSAID usage status (use vs. no use), but may have changed their NSAID dosage from T1 to T4.

Case ascertainment.

Participants had full colonoscopies at baseline (T0), 1 year (T1), and 4 years after randomization (T4). The colonoscopy at year 1 detected and removed any lesions missed at the baseline colonoscopy. There were 240 pathologically confirmed recurrent adenomas diagnosed at year 4 from the control arm of the PPT. A subset of recurrent cases were examined with either a) multiple adenoma recurrence or b) high-risk adenoma recurrence. 'Multiple recurrence' was defined as those individuals with >1 adenoma identified during their follow-up endoscopic procedure (n=102). 'High risk recurrence' was defined by 1 of 4 possible criteria: 1) adenoma diameter ≥1 cm, 2) evidence of high-grade dysplasia, 3) adenoma with >25% villous elements, or 4) greater than 2 adenomas present at T4 (n=67).

Blood sampling and MIC-1/GDF15 serum estimation.

All participants provided fasting venous blood samples at years 1 and 4 from which serum was separated and stored at −70°C. The time of sampling was between 366 days prior to and 391 days (mean=6 days; standard deviation=136 days) after the T1 colonoscopy and between 600 days before and 1184 days (mean = 140 days; standard deviation= 306 days) after the T4 colonoscopy. Serum MIC-1/GDF15 levels were determined using an enzyme immunoassay (24, 25).

Assessment of NSAID use.

Regular NSAID use was defined as those participants who reported either aspirin or non-aspirin NSAID use at least once per month (n=202) at study entry. The total dose of NSAID was assessed by an experienced interviewer at study entry, T1 and T4. NSAIDs included aspirin and other non-aspirin NSAIDs such as ibuprofen, naproxen, and indomethacin. Cyclooxygenase-2 specific inhibitors, were unavailable at the time of the study.

Statistical Analysis.

Statistical analyses were performed using STATA 11 (StataCorp, College Station, Tx, USA). Data presented as proportions, such as the baseline characteristics of study participants, stratified by adenoma recurrence, were compared by the χ^2 test. Serum MIC-1/GDF15 concentrations stratified by covariate data or adenoma recurrence were evaluated using the appropriate nonparametric statistical tests (Wilcoxon rank-sum or Kruskal-Wallis tests).

Odds ratios (ORs) and 95% confidence intervals (CIs) for adenoma recurrence were estimated within quartiles of serum MIC-1/GDF15 concentrations. Comparison of MIC-1/GDF15 serum levels with NSAID dosage was performed using simple linear regression. Multivariate logistic regression models included covariates that changed the OR for MIC-1/GDF15 by >10%, if they were significant predictors of adenoma recurrence ($p<0.05$ using the likelihood ratio test), or they had previously been documented to be associated with serum MIC-1/GDF15 levels (2, 3, 26–28). All models included age and gender. All statistical analyses were two-sided and differences were considered significant at $P<0.05$.

Results

Population characteristics

The baseline patient characteristics that exhibited a relationship with adenoma recurrence at 4 years (T4) after baseline (Table 1) were male gender ($P<0.01$), a history of multiple adenoma ($P<0.01$), and elevation in the waist-to-hip ratio ($P<0.01$). The proportion of regular NSAID users with adenoma recurrence at year 4 (64/204 [32%]) was significantly lower than the proportion of non-NSAID users (177/421 [42%], $P=0.01$). Additionally, NSAID dosage significantly and negatively correlated with adenoma recurrence ($P=0.04$) (Table 1).

MIC-1/GDF15 serum level predicts adenoma presence

At both T1 and T4, mean serum MIC-1/GDF15 levels differed significantly by polyp status with the lowest concentrations in polyp free subjects and the highest concentration in subjects with adenoma present at the time of the blood sampling (T1: 823 vs. 917 pg/ml, $P=0.02$ and T4: 928 vs. 1,020 pg/ml, $P=0.04$) (Table 2). At both T1 and T4, serum MIC-1/GDF15 concentrations also increased with age ($P<0.01$), waist-to-hip ratio ($P<0.01$), current smoking ($P<0.01$) and male sex ($P<0.01$) and history of multiple adenomas ($P<0.01$) (Table 2). Males had a significantly higher BMI ($P<0.01$), consumed more alcohol ($P<0.01$) and were more likely to have a smoking history ($P<0.01$). Serum MIC-1/GDF15 levels in patients who had had an adenoma removed were no different from patients who had had no adenoma detected at both T1 and T4 (T1: 823 vs. 885 pg/ml, $P=0.15$ and T4: 928 vs. 962 pg/ml, $P=0.9$) (Table 2). Patients with multiple adenomas had significantly higher serum MIC-1/GDF15 levels at T1 and T4 ($P=0.02$, $P<0.01$: respectively: Table-3). Additionally, serum MIC-1/GDF15 levels at T4 significantly increased with increasing numbers of adenomas present and high-risk adenoma recurrence ($P<0.01$; Table-3).

MIC-1/GDF15 levels are increased in NSAID users

Serum MIC-1/GDF15 concentrations were higher among NSAID users at both T1 and T4 (Table 2). However, this just failed to reach significance at T1 ($P=0.06$), while being highly significant at T4 ($P<0.01$; Table 2). The significant number of patients that had blood sampling with an adenoma present could have attenuated the relationship of MIC-1/GDF15 levels to NSAID use. When regular NSAID users were further stratified according to the presence or absence of an adenoma at the time of blood sampling, NSAID users at T1 and T4 with no adenoma present had significantly higher levels of serum MIC-1/GDF15 compared with subjects without a polyp who were not taking NSAIDs (T1: 882 vs.

805pg/ml, P<0.01 and T4: 1,044 vs. 860 pg/ml, P<0.01). In these patients at T1 (n=525) and T4 (n=534) serum MIC-1/GDF15 level was associated with the dose of NSAIDs used (T1, P<0.05; T4, P=0.03; linear regression).

NSAIDs reduce adenoma recurrence risk when MIC-1/GDF15 levels are increased

NSAID use is known to provide protection from adenoma recurrence in this cohort (Table-1) (29). Additionally, as demonstrated above, MIC-1/GDF15 serum levels were higher among subjects on NSAIDs at T1 and T4 with no adenoma present (Table-2). Further, serum MIC-1/GDF15 levels were significantly related to the dose of NSAIDs taken (P<0.05; linear regression). These results suggested that high serum MIC-1/GDF15 level, which is associated with NSAID use, could also be associated with a reduced risk of adenoma development. In the 'T1-adenoma free' subgroup, serum MIC-1/GDF15 was significantly higher in patients taking NSAIDs (805 vs. 882 pg/ml; P<0.01). In patients with elevated serum MIC-1/GDF15 levels (≥1200 pg/ml (1)) at T1 (n=139), NSAID use was associated with protection from adenoma recurrence (P=0.03), whilst NSAID use with no elevation of MIC-1/GDF15 (<1200 pg/ml; n=481), did not protect from recurrent adenoma (P=0.84). This suggested that a high adenoma free, NSAID associated, MIC-1/GDF15 serum level could identified subjects who were relatively protected from adenoma formation. Further examination of this phenomena indicated that those patients having a serum MIC-1/GDF15 level less than 1200 pg/ml were taking, on average, half the dose of NSAIDs than those patients with a higher serum MIC-1/GDF15 level (p<0.01). However, in multivariate logistic regression analysis NSAIDs protected against polyp recurrence independently of age, NSAID dosage and MIC-1/GDF15 level ≥1200 pg/ml (p=0.026) indicating a protective role for NSAID use, independent of MIC-1/GDF15. Therefore, both elevated polyp free MIC-1/GDF15 serum level, NSAID use and possibly NSAID dose, each, independently, are associated with reduced risk of recurrent adenoma in this cohort.

Serum MIC-1/GDF15 levels predict adenoma recurrence

From the above results, change in NSAID use and dose, as well as the time of blood sampling relative to polypectomy might significantly affect analyses examining whether MIC-1/GDF15 serum levels could be used to identify risk of adenoma recurrence. We therefore examined the 'Adenoma/NSAID appropriate' subgroup of 253 subjects where MIC-1/GDF15 serum level determination was appropriate with respect to polypectomy and NSAID usage status was the same at T1 and T4 (Table-4). Univariate analysis indicated that the top quartile of T4 MIC-1/GDF15 serum levels predicted adenoma recurrence (OR=3.8; 95%CI 1.4–10.4: P<0.01). Adjustment for NSAID use and dosage failed to attenuate the association of serum MIC-1/GDF15 to predict adenoma recurrence (Table-4). Similarly, adjustment for factors that might be related to serum MIC-1/GDF15 levels (Table-2), independent of NSAIDs, also failed to reduce this association. Indeed, in both cases the relationship of MIC-1/GDF15 serum level with adenoma recurrence appeared to strengthen (Table-4). Further adjustment for these factors, as well as the potential protective nature of polyp free MIC-1/GDF15 at T1, indicated that the risk of adenoma recurrence was more than 14 times more likely if the serum MIC-1/GDF15 serum level was in the top quartile at T4 (OR=14.7 95%CI 3.0–73; P<0.01).

Discussion

To our knowledge, this is the first study to report measurements of serum MIC-1/GDF15 in relation to NSAID use and adenoma presence / recurrence in prospectively followed, at risk patients. Consistent with data from experimental animals, we observed a clear association between elevated serum MIC-1/GDF15 concentrations, NSAID use and protection from adenoma recurrence. Further, and as previously reported (1) elevated serum MIC-1/GDF15 serum levels were associated with adenoma presence. Changes in serum MIC-1/GDF15 levels on serial measurements were also associated with adenoma recurrence.

The protective and predictive roles of MIC-1/GDF15 with respect to colonic adenomatosis might seem, at first glance, to be paradoxical. However, these findings are consistent with both our basic understanding of the role of MIC-1/GDF15 in polyposis from animal studies (16–18) and the change in serum MIC-1/GDF15 levels throughout the development of colon cancer in humans (1). MIC-1/GDF15 is produced by neoplastic colonic epithelium at a different stage of the disease process (30) and protects from colonic tumor formation in animal models (16–18) although the reasons underlying these changes are not clear. MIC-1/GDF15, like its relative TGF-β, seems to have a complex effect on tumour growth and development. In *in vitro* and *in vivo* experimental systems, MIC-1/GDF15 most frequently reduces tumour growth activity, but has also been reported to promote tumour growth and spread under some circumstances (reviewed by Breit et al. (30)) These factors are likely to contribute to the complex relationship between serum MIC-1/GDF15 serum levels and the presence, recurrence and/or protection from colonic polyposis

As far as we are aware MIC-1/GDF15 is the first serum marker having any relationship to the presence of colonic adenomas with potential clinical utility. Because there are no clinically useful serum markers of premalignant colonic disease, available cohorts studying colonic polyposis are limited. This cohort was studied because it is the only cohort to have prospectively evaluated at risk patients and collected serum that we were aware of. However, even the analysis of this cohort was significantly limited by the timing of blood sampling. Many subjects had their blood taken while a polyp was present at T1 or after it was removed at T4 leading to exclusion from the analysis of serial MIC-1/GDF15 serum levels. Whilst this issue was managed by exclusion of inappropriately timed samples, it resulted in a significant reduction in the number of subjects available to assess the utility of serum MIC-1/GDF15 measurement in the prediction of recurrent adenomas. This selection procedure may have also introduced bias from unappreciated sources. Another limiting factor was the large variation in the time of serum MIC-1/GDF15 measurement with respect to colonoscopy. This could not be adjusted for in our models examining serial MIC-1/GDF15 measurement, as those patients having recurrence were identifiable by blood sampling prior to polypectomy, while patients with no recurrence were sampled before and after their colonoscopy at T4. This variation in the timing of blood sampling, combined with the strong relationship between age and MIC-1/GDF15, probably contributed the relatively small differences in serum MIC-1/GDF15 serum levels between polyp free and adenoma relapse states between groups. However, because of the range of serum MIC-1/GDF15 levels, these differences may be larger in the individual undergoing serial sampling. Despite these major limitations, in the current study we were able to demonstrate a relationship of

serum MIC-1/GDF15 serum levels with the presence and recurrence of a colonic adenoma. Finally, the PPT cohort used in the current study was 92% Caucasian; and thus, results from this study may not be generalizable to more ethnically diverse populations. However, the results as they stand suggest that an initial polyp free serum MIC-1/GDF15 level, defined by colonoscopy in this case, with repeated serum MIC-1/GDF15 estimation over time, might be a clinically useful screening strategy for the detection of recurrent or initial colonic polyps.

Additional findings suggest that MIC-1/GDF15 would preferentially detect premalignant colonic adenomas requiring intervention. MIC-1/GDF15 serum levels were significantly related to the number of adenomas present in the starting cohort of 623 patients (Table-3) and were further elevated in subjects with high-risk recurrences or multiple adenomas present. Therefore it is possible that raised serial MIC-1/GDF15 levels could indicate clinically relevant adenoma recurrences in preference to low-risk adenoma recurrence. Indeed, as the study progressed the relationship of MIC-1/GDF15 serum levels to adenoma presence appeared to strengthen. Perhaps this was because there were adenomas that were developing or missed at T1 colonoscopy and became apparent three years later. Tandem back-to-back colonoscopic studies indicating that up to 27% of adenomas can be missed (31). With this in mind, it seems likely that a significant number of polyps would have been missed at repeat colonoscopy at T1 and be more easily detected at T4.

In this cohort, Tangrea and colleagues (29) reported a 23% reduction in the risk of adenoma recurrence with regular NSAID use. In our examination of the cohort, subjects taking NSAIDs who did not have elevated serum MIC-1/GDF15 levels had the same risk of adenoma recurrence as patients not taking NSAIDs, suggesting that, as in animal models (16, 18), MIC-1/GDF15 might mediate part of the protection from adenoma afforded by NSAID use. The complex interactions between NSAID use, adenoma recurrence and serum MIC-1/GDF15 level make it difficult to interpret the adjustment for NSAIDs in multivariate logistic regression as they are interrelated and might lead to ‘over fitting’ of regression models. However, univariate regression indicated a significant relationship which, when adjusted for potentially confounding factors, only strengthened. This situation might have occurred because MIC-1/GDF15 serum levels are related to most risk factors for colonic polyposis and the development of cancer (Table-2). While potentially affecting multivariate regression analysis, it would seem that such relationships support, rather than detract from, the likelihood that serum MIC-1/GDF15 serum levels are related to NSAID use and adenoma formation. Supporting MIC-1/GDF15 as a mediator of NSAID protection from adenomas is the finding that serum MIC-1/GDF15 levels were correlated to NSAID dose at both T1 and T4. Additionally, those subjects with low MIC-1/GDF15 using NSAIDs were taking about half the dose of those subjects that had serum levels (≥1200 pg/ml). While data showing MIC-1/GDF15 protects from, and is produced by, colonic adenomas might seem paradoxical, they are consistent with animal data showing that MIC-1/GDF15 mediates the protective actions of NSAIDs against colonic polyposis (16, 18). Interestingly, NSAID induced cell cycle arrest in ovarian cancer cells is also dependent on MIC-1/GDF15 (14).

The apparent paradoxical actions of MIC-1/GDF15 are not unprecedented, as a close relative, TGF-β, is produced by normal and neoplastic colonic epithelium, and has similar anti-neoplastic as well as tumor promoting actions in the colon (32). Early studies of MIC-1/

GDF15 suggested that it has anticancer activity and induced apoptosis of cancer cells *in vitro*. However, there is also evidence that MIC-1/GDF15 may participate in tumor progression. The anti-tumourigenenic effect of MIC-1/GDF15 is best demonstrated in transgenic or induced animal models of cancer outlined above. A limited number of tumor xenograft studies also show that MIC-1/GDF15 over expression in HCT-116 colon resulted in reduced tumor size when engrafted in nude mice (11, 33). A glioblastoma cell line, unresponsive to MIC-1/GDF15 *in-vitro*, completely failed to grow as a tumor xenograft in nude mice when transfected with MIC-1/GDF15 (34). This suggests MIC-1/GDF15 may have significant paracrine effects that modulate the tumor environment. One potential paracrine mechanism could be anti-angiogenic activity that has been documented both *in vitro* and *in vivo* (35).

A number of *in vitro* studies have been performed to gain an understanding of the molecular pathways and mechanisms utilised by MIC-1/GDF15. For example, many dietary compounds associated with neoplastic cell growth suppression (Kim JS et al., 2005 Lee SH et al., 2005) induce MIC-1/GDF15 expression (36–38). Many studies have also suggested that MIC-1/GDF15 induces tumor apoptosis (11, 39, 40). However, in one study MIC-1/GDF15 expression was associated with a more invasive gastric cancer cell line phenotype and could induce increased gastric cancer cell invasion *in vitro*. This appeared to be due to MIC-1/GDF15 increasing expression of the urokinase type plasminogen activator (uPA) and the uPA receptor (uPAR) (41). Thus while most studies highlight an anti-tumorigenic role for MIC-1/GDF15, some suggest support for tumor growth and/or dissemination.

In conclusion, our data demonstrate that serum MIC-1/GDF15 concentrations are associated with known modifiers of risk of colorectal cancer, including NSAID use, and suggest a biological role for MIC-1/GDF15 in suppressing early colonic neoplasia. These data suggest that inducing an ‘appropriate’ rise in serum MIC-1/GDF15 levels could optimize NSAID prevention of colonic neoplasia. Additionally, where polyps are present, serum MIC-1/GDF15 levels appear to be a biomarker of adenomatous polyp burden and are related to adenoma recurrence in this cohort. Despite the limitations of the cohort, these data are encouraging. They suggest that prospective clinical trials specifically designed to evaluate MIC-1/GDF15 are justified and required to determine the optimal strategy for the use of serum MIC-1/GDF15 level measurement for the prevention of colon cancer.

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Table 1.

Characteristics of participants in Polyp Prevention Trial by adenoma recurrence.[a]

	Total		No Recurrence		Recurrence		
Baseline Characteristics	**N**	**%**	**N**	**%**	**N**	**%**	**P-Value**
Age							
Quartile 1 (35–53)	150	24	100	16	50	8	
Quartile 2 (54–62)	159	26	93	15	66	11	
Quartile 3 (63–70)	163	26	104	17	59	9	
Quartile 4 (71–86)	151	24	86	14	65	10	**P = 0.2620**
Sex							
Male	382	61	209	34	173	28	
Female	241	38	174	28	67	11	**P < 0.0001**
Race							
Caucasian	572	92	354	57	218	35	
Other	51	8	29	5	22	4	**P = 0.4820**
Waist to Hip Ratio							
Tertile 1 (0.62–91)	204	33	146	23	58	9	
Tertile 2 (0.92–0.98)	205	33	114	18	91	15	
Tertile 3 (0.99–1.51)	204	33	115	18	89	14	**P = 0.0014**
Smoking History							
No	546	88	336	54	210	34	
Yes	77	12	210	34	30	5	**P = 0.9331**
Family History of CRC							
No	173	28	103	17	70	11	
Yes	450	72	280	45	170	27	**P = 0.5382**
History Multiple adenoma							
No	410	66	283	45	127	20	
Yes	213	34	100	16	113	18	**P < 0.0001**
Education Status							
<=High School	154	23	96	15	58	9	
>High School	469	75	287	46	182	29	**P = 0.8002**
Regular NSAID use[b]							
No	421	68	245	39	176	28	
Yes	202	32	138	22	64	10	**P= 0.0144**
NSAID dose (mg per day)[b]							
None	421	68	245	39	176	28	
0–143	70	11	47	8	23	4	
144–325	77	12	49	9	28	4	
326–4725	55	9	42	7	13	2	**P= 0.0363**
Alcohol Intake (grams per day)							
None	251	40	165	26	86	13	

	Total		No Recurrence		Recurrence		
Baseline Characteristics	**N**	**%**	**N**	**%**	**N**	**%**	**P-Value**
Tertile 1 (0.3–3.99)	139	22	83	13	56	9	
Tertile 2 (2.00–12.99)	107	17	66	10	41	7	
Tertile 3 (13.00–139.00)	120	19	65	10	55	9	**P = 0.0684**

[a] Any Adenoma recurrence at T4 vs. no adenoma recurrence at T4.

[b] Defined as Regular NSAID use and dose at study entry.

Table 2.

Geometric means of MIC-1/GDF15 levels at T1 and T4 by patient characteristics

	Serum MIC-1/GDF15 levels T1 (pg/ml)					Serum MIC-1/GDF15 levels T4 (pg/ml)				
Patient characteristics	**N**	**%**	**Mean**	**SEM**	**P-Value**	**N**	**%**	**Mean**	**SEM**	**P-Value**
Total	623	100	848	16		623	100	949	19	
Polyp status										
No polyp	417	67	823	29		383	61	928	44	
Polyp removed	111	18	885	49		152	24	962	52	
Polyp present	95	15	917	42	**P = 0.0255**	88	14	1,020	51	**P = 0.0433**
Age										
Quartile 1 (35–53)	150	24	575	17		150	24	608	20	
Quartile 2 (54–62)	159	26	771	24		159	26	857	28	
Quartile 3 (63–70)	163	26	995	31		163	26	1,165	41	
Quartile 4 (71–86)	151	24	1,160	38	**P < 0.0001**	151	24	1,317	45	**P < 0.0001**
Sex										
Male	382	61	906	23		382	61	1,011	27	
Female	241	37	763	21	**P < 0.0001**	241	37	763	27	**P < 0.0001**
Waist to Hip Ratio										
	10	2				10	2			
Tertile 1 (0.62–0.91)	204	33	740	22		204	33	740	22	
Tertile 2 (0.92–0.98)	205	33	877	29		205	33	877	29	
Tertile 3 (0.99–1.51)	204	33	935	30	**P < 0.0001**	204	33	935	30	**P < 0.0001**
Smoking Status										
Never or Never Regular	257	41	761	22		257	41	761	22	
Former	289	46	885	24		289	46	885	24	
Current	77	12	1,037	49	**P < 0.0001**	77	12	1,037	49	**P < 0.0001**
Regular NSAID use[a]										
	3	0				1	0			
No	386	62	821	19		359	58	885	24	
Yes	234	38	891	28	**P = 0.0554**	263	42	1,038	31	**P = 0.0001**
History of multiple adenoma										
No	339	54	779	24		339	54	873	42	
Yes	284	46	938	40	**P < 0.0001**	284	46	1,048	45	**P < 0.0001**

[a]Regular NSAID use (<1 per month) vs. no regular NSAID use (<1 per month) reported at years 1 (T1) and 4 (T4), respectively

Table 3.

Geometric means of MIC-1/GDF15 levels at T1 and T4 by presence of a recurrent adenoma at T1 and T4

	Serum MIC-1/GDF15 levels T1 (pg/ml)					Serum MIC-1/GDF15 levels T4 (pg/ml)				
	N	**%**	**Mean**	**SEM**	**P-Value**	**N**	**%**	**Mean**	**SEM**	**P-Value**
Adenoma recurrence										
No adenoma	417	67	823	15		383	61	928	44	
Adenoma recurrence	206	33	900	33	**P = 0.0118**	240	39	983	38	**P = 0.0254**
Present at sampling	*95*	*15*	*917*	*28*	***P = 0.0124***	*88*	*14*	*1,020*	*51*	***P = 0.0188***
Absent at sampling	*111*	*18*	*885*	*49*	***P = 0.1482***	*152*	*24*	*962*	*52*	***P = 0.8524***
Multiple adenoma recurrence										
No adenoma	417	67	823	15		383	61	928	44	
Multiple adenoma recurrence	76	12	939	48	**P = 0.0208**	102	16	1,078	54	**P = 0.0006**
Present at blood sampling	*29*	*5*	*954*	*82*	***P = 0.0474***	*37*	*6*	*1,145*	*73*	***P = 0.0024***
Absent at blood sampling	*47*	*8*	*929*	*94*	***P = 0.1329***	*65*	*10*	*1,042*	*73*	***P = 0.0261***
Number of recurrent adenoma (Adenoma present at blood sampling)										
0	417	67	823	15		383	61	928	44	
1	66	11	901	52		51	8	937	69	
2	17	3	896	91		26	4	1,138	85	
3	5	1	904	204		7	1	1,149	203	
≥4	7	1	1,155	142	**P = 0.0758**	4	1	1,187	235	**P = 0.0492**
Number of recurrent adenoma (Adenoma removed prior to blood sampling)										
0	417	67	823	15		383	61	928	44	
1	64	10	854	49		87	14	905	73	
2	24	4	967	164		27	4	912	101	
3	12	2	815	101		22	4	1,075	135	
≥4	11	2	982	42	**P = 0.4516**	16	3	1,249	153	**P = 0.0428**
High risk recurrence at T4										
No adenoma						383	61	928	44	
Advanced recurrence						67	11	1,105	60	**P = 0.0022**

Table 4.

Risk of adenoma recurrence by quartiles of serum MIC-1/GDF15 levels at T4 adjusted for factors influencing MIC-1/GDF15 level and the protective effect of MIC-1/GDF15 in the 'Adenoma/NSAID appropriate' group.

	Any Adenoma Recurrence (n=253)[a]		
Regression Model	**OR***	**95% CI**	**P-Value**
Univariate			
Quartile 2 (612 – 831 pg/mL)	2.3	0.8 – 6.6	0.131
Quartile 3 (832 – 1158 pg/mL)	3.3	1.2 – 6.4	0.022
Quartile 4 (1159 – 6520 pg/mL)	3.8	1.4 – 10.4	0.009
Multivariate			
Adjustment for NSAID use			
Quartile 2 (612 – 831 pg/mL)	2.7	0.9 – 7.9	0.079
Quartile 3 (832 – 1158 pg/mL)	4.4	1.5 – 12.9	0.007
Quartile 4 (1159 – 6520 pg/mL)	5.2	1.8 – 15.1	0.002
NSAID use (Yes)	0.3	0.2 – 0.8	0.008
Change in NSAlD dose (100 mg)	0.9	0.8 – 1.0	0.023
Adjustment for non-NSAID factors associated with serum MIC-1/GDF15 level			
Quartile 2 (612 – 831 pg/mL)	3.0	0.9 – 9.5	0.069
Quartile 3 (832 – 1158 pg/mL)	4.8	1.4 – 16.8	0.013
Quartile 4 (1159 – 6520 pg/mL)	5.9	1.6 – 21.8	0.008
Sex (M)	1.8	0.7 – 4.5	0.264
Waist-to-hip T1 (cm/cm)	1.3	0.0 – 105	0.877
Age (10 years)	0.7	0.4 – 1.1	0.111
Alcohol use T4 (10g/day)	0.9	0.7 – 1.1	0.712
History of multiple adenoma (yes)	2.1	1.1 – 4.1	0.040
Time T1 to T4 (1 year)	0.3	0.0 – 3.4	0.674
Adjustment for significant NSAID and non-NSAID factors			
Quartile 2 (612 – 831 pg/mL)	3.7	1.1 – 12	0.035
Quartile 3 (832 – 1158 pg/mL)	5.8	1.6 – 21	0.008
Quartile 4 (1159 – 6520 pg/mL)	7.5	2.0 – 29	0.003
Sex (M)	2.1	1.0 – 4.3	0.045
Age (10 years)	0.7	0.5 – 1.1	0.116
History of multiple adenoma (yes)	1.9	0.9 – 3.7	0.078
NSAID use (Yes)	0.4	0.2 – 0.8	0.011
Change in NSAID dose (100 mg)	0.9	0.8 – 1.0	0.025
Additional adjustment for protective effect of MIC-1/GDF15 at T1			
MIC-1/GDF15 T4			
Quartile 2 (612 – 831 pg/mL)	4.1	1.2 – 14	0.025
Quartile 3 (832 – 1158 pg/mL)	7.7	2.0 – 30	0.003
Quartile 4 (1159 – 6520 pg/mL)	14.7	3.0 – 73	0.001
Sex (M)	2.1	1.0 – 4.4	0.038
Age (10 year)	0.8	0.5 – 1.2	0.231

	Any Adenoma Recurrence (n=253)[a]		
Regression Model	**OR***	**95% CI**	**P-Value**
History of multiple adenoma (yes)	2.0	1.0 – 3.9	0.063
NSAID use (Yes)	0.4	0.2 – 0.8	0.012
Change in NSAID dose (100 mg)	0.9	0.8 – 1.0	0.017
MIC-1/GDF15 T1 (1000 pg)	0.5	0.2 – 1.3	0.152

[a] Any adenoma recurrence at T4 (n=48) vs. no recurrence at T4 (n=205)