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# Prognostic significance of autocrine motility factor receptor expression by colorectal cancer and lymph node metastases

Yasmin Halwani, M.D.[a], Liliana D. Kojic, Ph.D.[b], Simon K. Chan, M.Sc.[c], Terry P. Phang, M.D., M.Sc.[a], Hamid Masoudi, M.D.[d], Steven J. M. Jones, Ph.D.[c], Ivan R. Nabi, Ph.D.[b], Sam M. Wiseman, M.D.[a,*]

[a]*Department of Surgery, St. Paul's Hospital,* [b]*Department of Cellular and Physiological Sciences,* [c]*Department of Medical Genetics, and* [d]*Department of Pathology and Laboratory Medicine, St. Paul's Hospital, University of British Columbia, Vancouver, British Columbia, Canada*

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**Abstract**

**BACKGROUND:** Autocrine motility factor receptor (AMFR) has been linked to metastasis and tumorigenicity. The aim of this study was to evaluate expression and prognostic significance of AMFR in colorectal carcinoma.

**METHODS:** AMFR expression was evaluated in 127 colon cancer specimens, 131 rectal cancer specimens, and 47 colonic and 25 rectal corresponding lymph node metastases. Clinicopathological correlates of prognostic significance were established by univariate and multivariate analysis. Spearman's correlation determined the association of expression between cancers and their metastases.

**RESULTS:** AMFR was over-expressed by 22% of colon cancers and 18% of rectal cancers. AMFR over-expression correlated significantly with improved disease-free survival (DFS) ($P < .05$) in colon cancer and decreased DFS in corresponding nodal metastases. In rectal cancer, AMFR over-expression significantly correlated with decreased overall survival, DFS, and disease-specific survival ($P < .001$, $P = .031$, $P = .005$, respectively) and decreased overall survival in corresponding metastases.

**CONCLUSION:** AMFR may serve as a molecular prognosticator for colon cancer and rectal cancer.


Colorectal cancer is the second leading cause of cancer death among American men and women combined. In 2014, the American Cancer Society estimated that approximately 136,830 Americans will be diagnosed with colorectal cancer, and 50,310 will die from this disease. These cancer-related deaths occur despite advances in screening, surgical techniques, and adjuvant chemotherapy. Development of regional and distant metastases is the major determinant of disease prognosis and thus dictates subsequent therapy. This has prompted increased interest in molecular profiling of colorectal cancer to better predict disease prognosis, and potentially identify targets for anticancer drugs.

The autocrine motility factor receptor (AMFR) represents a potential molecular prognosticator and target for cancer treatment. AMFR is a 78 kDa (gp78) glycoprotein that was

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* Corresponding author. Tel.: +1-604-806-9108; fax: +1-604-806-9957.

E-mail address: smwiseman@providencehealth.bc.ca

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first identified in metastatic B16-F1 melanoma cells.[1] It is a 643 amino acid transmembrane protein coding for an E3 ubiquitin ligase that plays a critical role in endoplasmic reticulum-associated degradation (ERAD), targeting misfolded or functionally denatured proteins for proteasomal degradation. AMFR is localized to the smooth endoplasmic reticulum and also at the plasma membrane, where it functions as a cytokine receptor that stimulates cell motility on binding to its ligand autocrine motility factor (AMF).[2]

AMF is equivalent to phosphoglucose isomerase, a glycolytic enzyme that plays an essential role in gluconeogenesis[3] and, on nonclassical secretion by tumor cells, functions as a cytokine.[2,4] AMF stimulates tumor cell motility inducing focal adhesion reorganization and loss of E-cadherin.[5,6] Using a knockdown approach, AMF was shown to be required for epithelial-to-mesenchymal transition in lung fibrosarcoma and breast cancer cells.[4,7] Over-expression of AMF induces cell motility, cell transformation and tumorigenicity in NIH-3T3 fibroblasts,[8] tumor growth and metastases in PaCa-2 pancreatic tumor cells,[5] and epithelial-to-mesenchymal transition in dysplastic MCF10A mammary epithelial cells.[9] AMF also exhibits anti-apoptotic activity and chemoprotective ability, promoting tumor cell survival via activation of PI3K/Akt signaling.[8,10,11] AMF also protects against the ER stress response and associated apoptosis through both PI3K signaling and dampening of ER calcium release.[12]

Several studies have reported that AMFR is either absent or expressed at significantly lower levels in normal tissue adjacent to bladder, colorectal, gastric, cutaneous, and esophageal cancers.[13–17] Furthermore, several studies have also suggested that AMFR expression correlates with advanced disease stage and decreased patient survival for several human cancer types including lung, esophagus, gastric, colorectal, hepatic, cutaneous, breast, and thymic malignancies.[15,18–23] Given its role in tumor progression and metastasis development,[23] AMFR may represent an attractive potential candidate for targeted cancer therapy. Importantly, the raft-dependent AMFR-mediated internalization of AMF is selective for metastatic tumor cells and may represent an effective drug delivery agent for cancer.[11,24]

To date, there has only been a single study evaluating AMFR expression in colorectal cancer.[16] In this study, we examined the expression of AMFR in a population of individuals who underwent resection of colon cancer or rectal cancer with a curative intent. The objective of this study was to separately evaluate the frequency of AMFR expression by colon and rectal cancer, to correlate AMFR expression with clinicopathological prognosticators and patient outcomes, and to evaluate AMFR expression in lymph node metastases.

## Patients and Methods

This is a single-center retrospective cohort study of individuals undergoing surgery for colorectal cancer between September 1997 and September 2005. There were 143 colon cancer patients and 130 rectal cancer patients identified from a database that was prospectively maintained by one of the authors (T.P.). The study was approved by our Research Ethics Board. Cancers 15 cm or higher above the anus were considered as colon cancer and cancers below this level were considered as rectal cancers. Only patients who underwent complete resection and had pathology specimens available for tissue microarray (TMA) construction were included in the study population. Patients who had a second colonic primary cancer or a recurrence of a previously resected cancer were excluded from the study population. Further exclusion criteria for this study included individuals who were operated on with a palliative intent, individuals with advanced disease or a T4 cancer, individuals undergoing emergency colon or rectal surgery, and those individuals who were lost to clinical follow-up. For survival analysis, patients who died perioperatively (defined as mortality within 30 days of surgery) were also excluded from the study population. Survival outcomes were calculated based on a median follow-up of 3.6 and 4.9 years for the colon and rectal cancer patients, respectively. All patients were followed up in accordance with the British Columbia Cancer Agency provincial guidelines for colorectal cancer patient follow-up.

Colon and rectal cancer TMAs were constructed using cancer specimens from a cohort of 127 colon cancer and 131 rectal cancer patients. Furthermore, lymph node metastases specimens from 47 colon and 25 rectal cancer patients, respectively, were also included in the TMA. TMAs were constructed as has been previously described.[25] AMFR expression was evaluated by immunohistochemistry using the 3F3A anti-AMFR rat IgM monoclonal antibody.[26] Briefly, the TMA blocks were cut into 4 mm sections using a Leica microtome (Leica Microsystems, Inc, Richmond Hill, ON) and the sections were transferred to adhesive-coated slides for immunohistochemical staining. As has been previously described, anti-AMFR (3F3A) mAb dilutions were validated using cores prepared from colon cancer cell pellets (HT-29, HCT-116, and Caco-2) to determine optimal AMFR detection, and compared with expression determined by flow cytometry and western blotting.[11] Sample TMA colon cancer cores exhibiting AMFR expression are shown in Fig. 1.

The TMA sections stained with anti-AMFR antibody were scored by 2 pathologists blinded to all clinical data. Any inter-pathologist disagreement was resolved by a third pathologist. Scoring system AMFR expression levels were given binary designations, in which AMFR staining was regarded as positive or over-expressed, when greater than 10% of the cells examined showed cytoplasmic staining. Based on the scoring system used, this was the most clinically relevant and reproducible cutpoint for this marker. AMFR expression levels were then correlated with clinicopathological variables and survival outcomes.

The correlation of AMFR expression with clinicopathological colorectal cancer prognosticators was carried out by univariate analyses. The clinicopathological variables