Original Study

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Ataxia Telangiectasia Mutated Protein Loss and Benefit From Oxaliplatin-based Chemotherapy in Colorectal Cancer

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Abstract

Loss of ATM, a key protein regulating DNA repair increases sensitivity to DNA damaging agents such as oxaliplatin chemotherapy. We describe the prevalence of ATM IHC loss in a large cohort of patients with metastatic colorectal cancer and its correlation with clinical parameters such as association with other key biomarkers in colon cancer and survival.

Background: Loss of ataxia telangiectasia mutated (ATM), a key protein regulating DNA repair signaling, has been suggested to increase sensitivity to DNA damaging agents. We conducted a study analyzing the loss of ATM protein expression in colorectal cancer and correlated this with clinical outcomes. **Materials and Methods:** The clinical outcomes data and tumor samples from metastatic colorectal cancer patients referred to the Royal Marsden Hospital Drug Development Unit (United Kingdom) from 2012 to 2016 and providing consent for a molecular characterization study were analyzed. Immunohistochemistry (IHC) slides were assessed by a pathologist for nuclear staining intensity of ATM and semiquantitatively scored. ATM loss was defined as a nuclear H-score of ≤ 10. **Results:** Of 223 colorectal cancer samples, ATM IHC loss was identified in 17 (8%). ATM loss was independent of the *RAS* and *RAF* mutational status. ATM loss was associated with superior overall survival after first-line oxaliplatin-based therapy (49 vs. 32 months; hazard ratio [HR], 2.52) but not with irinotecan-based therapy (24 vs. 33 months; HR, 0.72). ATM loss was not prognostic for survival from the diagnosis (50 vs. 44 months; HR, 1.43). **Conclusion:** ATM could be considered a biomarker for the development of novel DNA repair targeting agents and treatment of colorectal cancer.

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Keywords: ATM, ATR, Colorectal cancer, DNA damage repair, Oxaliplatin

Introduction

Ataxia telangiectasia mutated (ATM) and ATM and rad3-related (ATR) kinase are key checkpoint proteins of the DNA damage response pathway.[1] Preclinical models have suggested that ATM-deficient cell lines are sensitized to DNA damaging agents, including platinum chemotherapy and ATR inhibitors.[2] We hypothesized from data from phase I clinical trials that ATM deficiency in colorectal cancer would portend sensitivity to DNA damaging chemotherapeutic agents such as oxaliplatin and irinotecan.[3,4] To the best of our knowledge, data on ATM protein

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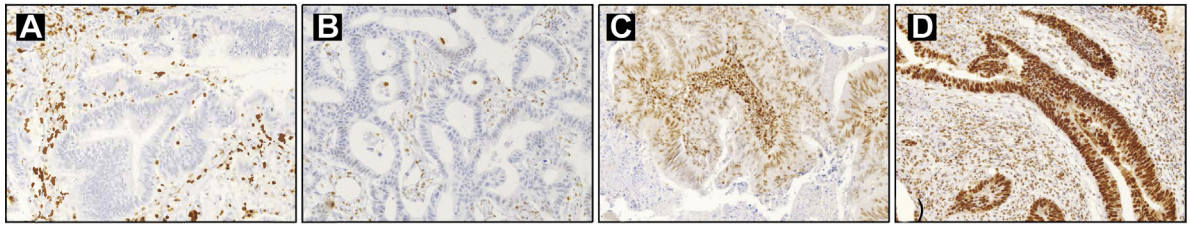
Figure 1 Ataxia Telangiectasia Mutated (ATM) Immunohistochemistry (IHC). IHC Images of Colorectal Cancer (Original Magnification, ×20) Showing Tumor Cell Nuclear H-score of (A) 0 (With Stromal Staining), (B) 10, (C) 90, and (D) 300



expression in colorectal cancer and corroboration with the clinical data and outcomes have not been reported previously.

Materials and Methods

Patient Cohort

Colorectal cancer patients who were referred to the Royal Marsden Hospital, Drug Development Unit (United Kingdom) from 2012 to 2016 and provided consent for participation to a molecular characterization study were identified as potential candidates. The clinical data for all patients were collected retrospectively from the electronic medical records. The information collected included patient demographic data and treatment history for colorectal cancer.

Ethics Approval and Consent to Participate

All patients provided written informed consent and were enrolled in accordance with institutional protocols approved by the Royal Marsden NHS Foundation Trust Hospital (London, UK) ethics review committee (approval no. CCR3171). The study was performed in accordance with the Declaration of Helsinki.

ATM Immunohistochemistry

Formalin-fixed, paraffin-embedded (FFPE) samples were obtained from primary tumor biopsy specimens, surgical resection specimens, and colorectal cancer metastases of the lymph nodes or viscera (needle biopsy samples). ATM protein expression was determined by immunohistochemistry (IHC) on 3- to 4-μM-thick FFPE sections using a rabbit monoclonal anti-ATM antibody Y170 at 1:400 (catalog no. ab32420; Abcam plc, Cambridge, UK).

The IHC slides were assessed by a pathologist, who was unaware of the patients' clinical characteristics, sequencing findings, and outcomes data. Nuclear staining was semiquantitatively assessed using an H-score formula: 3 times the percentage of strongly staining cells and 2 times the percentage of moderately staining cells, and the percentage of weakly staining cells, for a range of 0 to 300.[5] A dichotomous classification system was devised in which cases were considered ATM negative if they either showed a complete absence of ATM staining or weak intensity staining in $\leq$ 10% cancer cells (H-score $\leq$ 10).

Next Generation Sequencing

DNA was extracted from FFPE blocks using the FFPE Tissue DNA kit (Qiagen). DNA was quantified with the Quant-iT high-sensitivity PicoGreen double-stranded DNA Assay Kit (Invitrogen), and DNA quality control was assessed using the Illumina FFPE QC kit (catalog no. WG-321-1001), as described previously.[6]

Libraries were constructed from 40 ng of DNA using a customized Generead DNAseq Mix-n-Match, version 2, panel (Qiagen) covering 6025 amplicons (398,702 bp) across 113 genes.[7] The libraries were pooled and run using the MiSeq Sequencer (Illumina) at a minimum of 500×. FASTQ files were generated using the Illumina MiSeq Reporter, version 2.5.1.3. Sequence alignment and mutation calling were performed using BWA tools and the GATK variant annotator by the Qiagen GeneRead targeted exon enrichment panel data analysis web portal. Variant calls (variant call format files) were filtered using Galaxy tools. Somatic variant calls were manually inspected in an integrated genome viewer (Broad Institute, Cambridge, MA). The pathogenicity of the variants was assessed according to the reported data and public databases, including but not limited to, the ClinVar database (National Institutes of Health, Bethesda, MD).

Statistical Analysis

Survival was measured from the first date of treatment to the date of last contact or the date of death from any cause. The Kaplan-Meier product-limit method was used to estimate the duration of treatment, and overall survival (OS) and hazard ratios (HRs) were estimated using univariate Cox regression models. Patients who received oxaliplatin therapy in the adjuvant setting were excluded from analyses of first-line metastatic oxaliplatin survival but were included in the analyses for first-line irinotecan, because most of these patients had received adjuvant oxaliplatin. All tests were 2-sided, and $P \leq .05$ was considered to indicate statistical significance. Descriptive statistics and survival analyses were performed using Stata, version 13.1.

Results

Patient Characteristics

A total of 223 colorectal cancer patients were included in the present study and had tissue available for analysis for ATM IHC (Figure 1). The patient characteristics and treatment history are listed in Table 1. Adjuvant oxaliplatin was used in 66 patients (30%), of whom, 50 (76% of 66) were treated with first-line irinotecan and included in all analyses. Twelve patients treated with adjuvant oxaliplatin were rechallenged with the same agent in

Table 1 Patient Characteristics

Characteristic	n (%)
Total patients	223 (100)
Age, y	
Median	60
Range	19-86
Male gender	133 (60)
De novo metastatic disease from diagnosis	137 (61)
Interval from diagnosis to metastatic disease, mo	
Median	13
Range	2-170
Treatment lines, n	
Median	3
Range	1-7
First exposure to oxaliplatin	
Adjuvant/neoadjuvant	66 (30)
First-line metastatic	122 (55)
Second-line metastatic	29 (13)
Third-line and beyond	5 (2)
First-line metastatic oxaliplatin regimens[a]	
CAPOX	38 (31)
FOLFOX/bevacizumab	31 (25)
FOLFOX	21 (17)
CAPOX/bevacizumab	16 (13)
FOLFOX/cetuximab	9 (7)
First exposure to irinotecan	
First-line metastatic	82 (36)
Second-line metastatic	114 (51)
First-line metastatic irinotecan regimens[b]	
FOLFIRI/cetuximab	29 (35)
FOLFIRI/bevacizumab	26 (32)
FOLFIRI	10 (12)

Abbreviations: CAPOX = capecitabine, oxaliplatin; FOLFIRI = folinic acid, 5-fluorouracil, irinotecan; FOLFOX = folinic acid, 5-fluorouracil, oxaliplatin.
[a]Percentages computed from 122 patients.
[b]Percentages computed from 82 patients.

the first-line metastatic setting and were not included in the analyses involving oxaliplatin survival. Four patients were treated with oxaliplatin in the adjuvant setting and had received neither irinotecan nor oxaliplatin in the metastatic setting. Five patients were treated in the first-line setting with non-irinotecan/oxaliplatin–based regimens. No significant differences were found between the ATM-loss group and the ATM-proficient group regarding treatment with targeted therapy (Table 2).

ATM IHC Protein Loss, RAS, RAF, and Mismatch Repair Status

ATM IHC protein loss occurred in 17 cases (8%). A matched primary–metastatic sample was available for 19 cases and matched metastatic–metastatic samples were available for 4 cases. No discordance was seen in the ATM scores between the primary and metastatic sites or between the metastatic sites. Next generation sequencing (NGS) was performed on 213 samples (96%), of which 15 failed the quality control assessment, resulting in 198 samples with analyzable results. *ATM* mutations were detected in 10 cases (5%). Of these 10 cases, 5 were nonsynonymous mutations and 5 were truncating mutations. Of the 17 cases with ATM IHC loss, NGS was performed for 15, of which 4 (24%) were found to have *ATM* mutations.

RAS and *RAF* mutation information was available for 212 subjects. *RAS* mutations were reported in 119 cases (56%), with *KRAS* found in 113. *BRAF* mutations occurred in 12 (6%). Of the 17 patients with ATM IHC loss, 9 had mutations in *KRAS* (53%) and 1 in *BRAF* (6%). Mismatch repair (MMR) deficiency status data, detected through IHC, or microsatellite instability, detected by polymerase chain reaction, were available for 44 patients, of whom, 4 were found to be MMR deficient (9%; 4 of 44). One patient with absence of MLH1 and PMS2 staining on IHC was found to have an ATM IHC score of 0.

Table 2 Comparison of Therapies Received Stratified by ATM IHC Status

Therapy	ATM IHC Loss (n = 17)	ATM IHC Proficient (n = 206)
Adjuvant oxaliplatin	6 (35)	60 (29)
First-line metastatic oxaliplatin	9 (53)	113 (55)
With bevacizumab	3 (33% of 9)	45 (40% of 113)
With cetuximab	0 (0)	10 (9% of 113)
First-line metastatic irinotecan	7 (41)	75 (36)
Adjuvant oxaliplatin	5 (71% of 7)	45 (60% of 75)
With bevacizumab	3 (43% of 7)	33 (44% of 75)
With cetuximab	2 (29% of 7)	28 (37% of 75)

Data presented as n (%).
Abbreviations: ATM = ataxia telangiectasia mutated; IHC = immunohistochemistry.

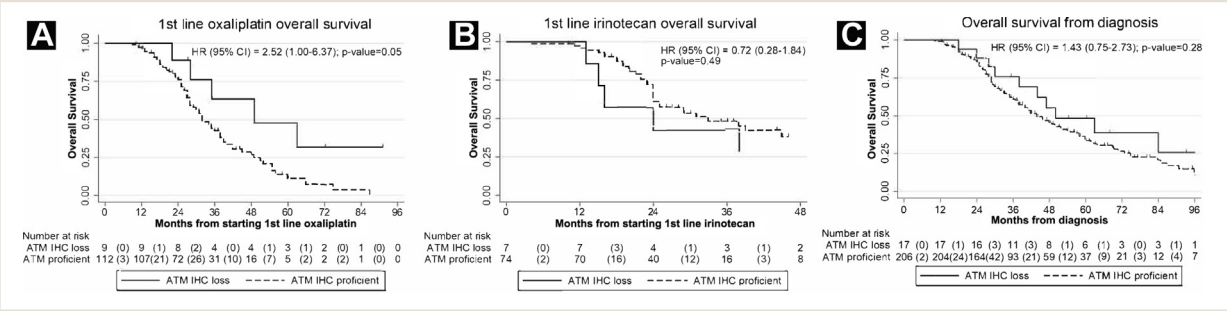
ATM Loss and Clinical Outcomes With Chemotherapy

Of the 121 patients who received oxaliplatin-based chemotherapy in the first-line metastatic setting, those with ATM IHC loss had superior OS (49 vs. 32 months; HR, 2.52; 95% confidence interval [CI], 1.00-6.37; $P = .05$). However, there was no statistically significant difference in OS among the 81 patients treated with first-line irinotecan-based therapy (24 vs. 33 months; HR, 0.72; 95% CI, 0.28-1.84; $P = .49$). Also, no difference was found in survival between first-line oxaliplatin therapy and first-line irinotecan therapy in the present cohort when ATM status was not considered (34 vs. 33 months; HR, 0.93; 95% CI, 0.62-1.39), consistent with historical data.[8] ATM IHC loss was not significantly associated with survival from diagnosis for the entire cohort (50 vs. 44 months; HR, 1.43; 95% CI, 0.75-2.73; $P = .28$). Kaplan-Meier plots are displayed in Figure 2.

Discussion

To date, precision medicine in colorectal cancer is largely based on negative predictive biomarkers such as *RAS* mutational status, precluding the use of anti-EGFR antibodies.[9] Therapies for other predictive biomarkers such as BRAF and HER2 are

Figure 2 Correlation Between Ataxia Telangiectasia Mutated (ATM) Loss and Survival With Oxaliplatin or Irinotecan. (A) Kaplan-Meier Plot of Overall Survival of Colorectal Cancer Patients Treated With First-line Oxaliplatin-based Chemotherapy. (B) Kaplan-Meier Plot of Overall Survival of Colorectal Cancer Patients Treated With First-line Irinotecan-based Chemotherapy. (C) Kaplan-Meier Plot of Overall Survival of Colorectal Cancer Patients From Diagnosis (Irrespective of Treatment)



CI = confidence interval; HR = hazard ratio; IHC = immunohistochemistry.

currently under development; BRAF and HER2 constitute ~10% of all metastatic colon cancer cases. In our study, we found that ATM protein occurs in 8% of colorectal cancer cases and appears to be independent of *RAS* and *RAF* mutational status. ATM loss has been described in various tumor groups. In gastric cancer, ATM loss occurs in ~20% of the population.[10] ATM was studied as a potential biomarker in gastric cancer for treatment with olaparib, a PARP (poly ADP-ribose polymerase) inhibitor. Although the results from an initial phase II study appeared promising,[11] those from a subsequent randomized phase III study were negative.[12] Biologically and clinically more relevant might be the synthetic lethality of ATM deficiency with ATR inhibition. ATR inhibitors are currently being evaluated, and an ATM assay could be useful to predict response to therapy. In a phase I study of VX-907, an ATR inhibitor, a patient with ATM loss colorectal cancer had a complete response to single-agent therapy.[4] Our results have shown that patients with ATM IHC loss colorectal cancer have a better outcome when treated with oxaliplatin-based therapy, consistent with preclinical data. As more DNA damage response targeting agents enter clinical trials, the use of ATM aberrancies as predictive biomarkers for these therapies will increase. It is possible that identification of ATM deficiency either through IHC or NGS will be incorporated into routine clinical biomarker testing, in addition to RAS, RAF and MMR in colorectal cancer in the future.

Clinical Practice Points

- DNA damage repair is emerging as a promising pathway to be targeted in several cancer subtypes such as breast and ovarian cancer.
- ATM and ATR are key proteins involved in DNA damage repair.
- ATM loss has been reported to occur in ~20% of cases of gastric cancer.
- Randomized studies with drugs targeting DNA damage repair have incorporated ATM as a potential biomarker to select for gastric cancer patients who might benefit from this form of therapy.
- The prevalence of ATM loss in colorectal cancer has previously never been described in a large cohort.
- In our cohort of > 200 patients, 8% were reported to have ATM loss as measured by protein expression, using immunohistochemistry.
- ATM loss appears to be independent of RAS and RAF mutational status.
- In our cohort, ATM loss in colorectal cancer resulted in superior overall survival when treated with first-line oxaliplatin chemotherapy.
- ATM could be considered as a potential predictive biomarker for the development of novel DNA repair-targeted therapy for patients with refractory colorectal cancer.

Conclusion

We have described the occurrence of ATM IHC loss in a large cohort of patients with colorectal cancer and its correlation with several other clinical relevant parameters such as *RAS* and *RAF* status. We have also demonstrated the improved clinical outcomes of patients with ATM IHC loss colorectal cancer when treated with oxaliplatin-based therapy. ATM could be considered as a potential predictive biomarker for the development of novel DNA repair targeted therapy for colorectal cancer.

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Disclosure

S.M., D.N.R., M.C.P., D.D., M.C., I.F., C.B., W.Y., A.F., R.C., G.B., D.R.P., N.T., J.M., S.C., and J.D.B. are employees of The Institute of Cancer Research (London, UK), which is a joint applicant for the patent entitled "DNA damage repair inhibitors for treatment of cancer," which includes the granted application US8143241. J.D.B. has served as an advisor for AstraZeneca, Medivation, Pfizer, Merck, Tesaro, and BioMarin. The remaining authors declare that they have no competing interests.

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