believed to play a role in the disease. Environmental components may include alterations in flora of the gut which are affected by exposure to ingested foods and medications.

[0009] IBD is associated with abdominal pain, vomiting, diarrhea, rectal bleeding, severe cramps, muscle spasms, weight loss, malnutrition, fever, and anemia. Patients with IBD may also suffer from skin lesions, joint pain, eye inflammation, and liver disorders, and children suffering from ulcerative colitis may suffer from growth defects. Although rarely fatal, these symptoms decrease quality of life for patients.

[0010] Thus, there is a pressing need to develop reliable methods of treating bowel diseases such as CRC and IBD. There is a further need to identify methods of treatment that provide effective and permanent relief from symptoms across a broad spectrum of patients and which are not associated with negative side effects or cycles of remission and/or inflammation.

SUMMARY

[0011] Described herein are antisense oligonucleotides that inhibit the expression of fragile X mental retardation protein (FMRP), and methods of using the same. In one aspect, the present disclosure provides an oligonucleotide for inhibiting the expression of fragile X mental retardation protein (FMRP), comprising a sequence selected from the group consisting of:

5′-CCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 1),
5′-CTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 2),
5′-TCCACCACCAGCTCCTCC-3′ (SEQ ID NO: 3),
5′-CTTCCACCACCAGCTCC-3′ (SEQ ID NO: 4),
5′-TCACCCTTTATCATCCTC-3′ (SEQ ID NO: 5),
5′-TCCACCACCAGCTCCTCCAT-3′ (SEQ ID NO: 6),
5′-ACTTCCACCACCAGCTCCTC-3′ (SEQ ID NO: 7),
5′-TTCCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 8),
5′-ACTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 9), and
5′-CTCACCCTTTATCATCCTCA-3′ (SEQ ID NO: 10), or a complement thereof.

[0011a] In another aspect, the present disclosure provides a pharmaceutically acceptable composition for inhibiting the expression of FMRP, comprising: (a) an oligonucleotide as described herein and (b) a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides use of an oligonucleotide as described herein in the

manufacture of a medicament for treating a bowel disease or treating or preventing a solid tumor, tumor invasion, or tumor metastasis. In another aspect, the present disclosure provides use of an antisense oligonucleotide or siRNA that inhibits the expression of fragile X mental retardation protein (FMRP) in the manufacture of a medicament for treating a bowel disease or treating or preventing a solid tumor, tumor invasion, or tumor metastasis.

[0011b] In some examples, the present disclosure provides a method of treating a bowel disease in a patient in need thereof comprising administering to the patient an effective amount of an antisense oligonucleotide that inhibits the expression of FMRP. In some examples, the bowel disease may be colorectal cancer or an inflammatory bowel disease such as Crohn's disease or ulcerative colitis. In some examples, the antisense oligonucleotide induces necroptosis.

[0012] In some examples , the present disclosure provides a method of treating a solid tumor, tumor invasion, or tumor metastasis in a patient in need thereof comprising administering to the patient an effective amount of an antisense oligonucleotide that inhibits the expression of FMRP. In some examples, the present disclosure provides a method of preventing or ameliorating tumor invasion or tumor metastasis. In certain examples, the present disclosure provides a method of preventing or ameliorating colorectal cancer tumor invasion or colorectal cancer tumor metastasis.

[0013] In some examples, the antisense oligonucleotide that inhibits the expression of FMRP comprises a sequence selected from the group consisting of:
5′-CCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 1),
5′-CTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 2),
5′-TCCACCACCAGCTCCTCC-3′ (SEQ ID NO: 3),
5′-CTTCCACCACCAGCTCC-3′ (SEQ ID NO: 4), and
5′-TCACCCTTTATCATCCTC-3′ (SEQ ID NO: 5), or a complement thereof.

[0014] In further examples, the antisense oligonucleotide that inhibits the expression of FMRP comprises a sequence selected from the group consisting of:
5′-TCCACCACCAGCTCCTCCAT-3′ (SEQ ID NO: 6),
5′-ACTTCCACCACCAGCTCCTC-3′ (SEQ ID NO: 7),
5′-TTCCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 8),
5′-ACTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 9), and
5′-CTCACCCTTTATCATCCTCA-3′ (SEQ ID NO: 10), or a complement thereof.

[0015] In some examples, the antisense oligonucleotide that inhibits the expression of FMRP consists of a sequence selected from the group consisting of:
5′-TCCACCACCAGCTCCTCCAT-3′ (SEQ ID NO: 6),
5′-ACTTCCACCACCAGCTCCTC-3′ (SEQ ID NO: 7),
5′-TTCCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 8),
5′-ACTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 9), and
5′-CTCACCCTTTATCATCCTCA-3′ (SEQ ID NO: 10), or a complement thereof.

[0016] In some examples, the antisense oligonucleotide may be an antisense oligonucleotide wherein at least one internucleoside linkage of the sequence is a phosphorothioate linkage, a phosphorodithioate linkage, a phosphotriester linkage, an alkylphosphonate linkage, an aminoalkylphosphotriester linkage, an alkylene phosphonate linkage, a phosphinate linkage, a phosphoramidate linkage, a phosphoromorpholidate linkage, a phosphoropiperazidate linkage, an aminoalkylphosphoramidate linkage, a thiophosphoramidate linkage, a thionoalkylphosphonate linkage, a thionoalkylphosphotriester linkage, a thiophosphate linkage, a selenophosphate linkage, or a boranophosphate linkage. In a particular example, at least one internucleoside linkage of the antisense oligonucleotide sequence is a phosphorothioate linkage. In some examples, all of the internucleoside linkages of the antisense oligonucleotide sequence are phosphorothioate linkages. In some examples, at least one nucleoside linkage of the sequence is a methylphosphonate linkage.

[0017] The number of nucleotides included in FMRP antisense oligonucleotides described herein may vary. For example, in some examples, the antisense oligonucleotide is from 20 to 40 nucleotides in length. In some examples, the antisense oligonucleotide is from 20 to 24 nucleotides in length.

[0018] In some examples, the FMRP antisense oligonucleotide induces necroptosis via activation of the receptor-interacting protein kinase 1 (RIP1 or RIPK1) - receptor-interacting protein kinase 3 (RIP3 or RIPK3) - mixed lineage kinase domain-like protein (MLKL) complex. For example, the FMRP antisense oligonucleotide may increase the expression of RIPK1.

[0019] In some examples, the FMRP antisense oligonucleotide comprises one or more ribonucleotides, one or more deoxyribonucleotides, or a mixture of ribonucleotides and deoxyribonucleotides.

[0020] In some examples, the FMRP antisense oligonucleotide comprises one or more modified nucleoside, for example, 5-methylcytidine, 5-methyl-2′-deoxycytidine, deoxycytidine, 5-methyl-2′-deoxycytidine 5′-monophosphate, or 5-methyl-2′-deoxycytidine-5′-monophosphorothioate. In certain examples, the FMRP antisense oligonucleotide comprises one or more modified nucleoside, for example, 2′-*O*-methylcytidine, 2′-*O*-methylguanosine, 2′-*O*-methylthymidine, 2′-*O*-methyluridine, or 2′-*O*-methyladenosine. In some examples, the FMRP antisense oligonucleotide comprises one or more modified nucleotide, for example, 5-methyl cytosine or 5-methylguanine. In some examples, the FMRP antisense oligonucleotides comprises one or more modified nucleotide, for example, 2′-*O*- (2-methoxyethyl) nucleosides, 2′-deoxy-2′-fluoro nucleosides, or 2′-fluoro-β-D-arabinonucleosides.

[0021] In some examples, the FMRP antisense oligonucleotide comprises bridged nucleic acids, locked nucleic acids (LNA), constrained ethyl (cET) nucleic acids, tricyclo-DNAs (tcDNA), 2′-*O*,4′-C-ethylene linked nucleic acids (ENA), or peptide nucleic acids (PNA).

[0022] In some examples, the FMRP antisense oligonucleotide is an FMRP siRNA, or a pharmaceutically acceptable salt thereof.

[0023] In some examples, the FMRP antisense oligonucleotide is administered to the patient enterally or pareneterally. For example, in some examples, the FMRP antisense oligonucleotide is administered to the patient orally, sublingually, gastrically, or rectally. In other examples, administration of the FMRP antisense oligonucleotide to the patient is intravenous, intratumoral, intrajejunal, intraileal, intracolonic, or intrarectal.

[0024] In particular examples, the FRMP antisense oligonucleotide of the present disclosure is for the treatment of a bowel disease in a human patient.

[0025] Also described herein is a pharmaceutically acceptable composition comprising an FMRP antisense oligonucleotide described herein and a pharmaceutically acceptable carrier. In some examples, the pharmaceutical composition is suitable for oral, sublingual, gastric, or rectal administration. In other examples the pharmaceutical composition is suitable for intravenous, intratumoral, intrajejunal, intraileal, intracolonic, or intrarectal administration.

[0026] Also described herein is use of an FMRP antisense oligonucleotide in the manufacture of a medicament for the treatment of a bowel disease. In some examples, the bowel disease is CRC, or an IBD such as Crohn’s disease or ulcerative colitis. In other examples, the medicament is administered to the patient enterally or parenterally. For

example, in some examples, the medicament is suitable for oral, sublingual, gastric, or rectal administration. In some examples, the medicament is suitable for intravenous, intratumoral, intrajejunal, intraileal, intracolonic, or intrarectal administration. In some examples, the medicament is for treatment of a bowel disease in a human.

[0027] In some examples, the present disclosure provides a use of an FMRP antisense oligonucleotide in the manufacture of a medicament for the treatment of a solid tumor, tumor invasion, or tumor metastasis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] FIG. 1A are immunohistological images showing staining for FMRP in human CRC samples (IgG=Isotype control; NC=colon sample of a normal control; P=peritumor sample; T=tumor sample). FIG. 1B is a line graph showing FMRP mRNA expression levels in peritumor (P) and CRC tumor samples (T) as determined by RT-PCR analysis (**$p<0.01$). FIG. 1C is a Western blot showing FMRP protein expression in paired peritumor (P) and CRC tumor samples (T) from CRC patients. FIG. 1D is a graph showing FMRP protein expression relative to β-actin expression in paired peritumor (P) and CRC tumor samples (T) as measured by quantitative densitometry of Western blot analyses (relative expression shown as arbitrary units (a.u.)).

[0029] FIG. 2A are endoscopic images of colons of untreated wild-type (WT) and FMR1 knock-out (KO) mice, or mice 21 weeks following intraperitoneal injection of Azoxymethane (AOM). FIG. 2B are bar graphs showing tumor load (left) and tumor size (right) in the colons

WHAT IS CLAIMED IS:

1. An oligonucleotide for inhibiting the expression of fragile X mental retardation protein (FMRP), comprising a sequence selected from the group consisting of:
5′-CCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 1),
5′-CTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 2),
5′-TCCACCACCAGCTCCTCC-3′ (SEQ ID NO: 3),
5′-CTTCCACCACCAGCTCC-3′ (SEQ ID NO: 4),
5′-TCACCCTTTATCATCCTC-3′ (SEQ ID NO: 5),
5′-TCCACCACCAGCTCCTCCAT-3′ (SEQ ID NO: 6),
5′-ACTTCCACCACCAGCTCCTC-3′ (SEQ ID NO: 7),
5′-TTCCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 8),
5′-ACTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 9), and
5′-CTCACCCTTTATCATCCTCA-3′ (SEQ ID NO: 10), or a complement thereof.

2. The oligonucleotide of claim 1, wherein the oligonucleotide comprises ribonucleotides, deoxyribonucleotides, or a combination of ribonucleotides and deoxyribonucleotides.

3. The oligonucleotide of claim 1 or 2, wherein the oligonucleotide comprises one or more modified nucleoside selected from the group consisting of 5-methylcytidine, 5-methyl-2′-deoxycytidine, deoxycytidine, 5-methyl-2′-deoxycytidine 5′-monophosphate, and 5-methyl-2′-deoxycytidine-5′-monophosphorothioate, 2′-*O*-methylcytidine, 2′-*O*-methylguanosine, 2′-*O*-methylthymidine, 2′-*O*-methyluridine, and 2′-*O*-methyladenosine, 5-methyl cytosine and 5-methylguanine, 2′-*O*-(2-methoxyethyl) nucleosides, 2′-deoxy-2′-fluoro nucleosides, and 2′-fluoro-β-D-arabinonucleosides.

4. The oligonucleotide of any one of claims 1 to 3, wherein the oligonucleotide comprises one or more modified nucleotide selected from the group consisting of bridged nucleic acids, locked nucleic acids (LNA), constrained ethyl (cET) nucleic acids, tricyclo-DNAs (tcDNA), 2′-*O*,4′-C-ethylene linked nucleic acids (ENA), and peptide nucleic acids (PNA).

5. The oligonucleotide of any one of claims 1 to 4, wherein the oligonucleotide comprises at least one internucleoside linkage selected from the group consisting of a

phosphorothioate linkage, a phosphorodithioate linkage, a phosphotriester linkage, an alkylphosphonate linkage, an aminoalkylphosphotriester linkage, an alkylene phosphonate linkage, a phosphinate linkage, a phosphoramidate linkage, a phosphoromorpholidate linkage, a phosphoropiperazidate linkage, an aminoalkylphosphoramidate linkage, a thiophosphoramidate linkage, a thionoalkylphosphonate linkage, a thionoalkylphosphotriester linkage, a thiophosphate linkage, a selenophosphate linkage, and a boranophosphate linkage.

6. The oligonucleotide of claim 5, wherein the oligonucleotide comprises at least one phosphorothioate linkage.

7. The oligonucleotide of claim 5, wherein all internucleoside linkages of the oligonucleotide are phosphorothioate linkages.

8. The oligonucleotide of any one of claims 1 to 7, wherein the oligonucleotide is capable of inducing necroptosis.

9. The oligonucleotide of any one of claims 1 to 8, wherein the oligonucleotide is capable of increasing activation of one or more kinases selected from the group consisting of: receptor interacting protein kinase 1 (RIPK1), receptor interacting protein kinase 3 (RIPK3), and mixed lineage kinase domain-like protein (MLKL).

10. The oligonucleotide of any one of claims 1 to 9, wherein the oligonucleotide is an antisense oligonucleotide or part of an siRNA, or a pharmaceutically acceptable salt thereof.

11. The oligonucleotide of claim 10, wherein the oligonucleotide is part of an siRNA, and wherein at least one cytidine of the oligonucleotide is 5-methylcytidine.

12. A pharmaceutically acceptable composition for inhibiting the expression of FMRP, comprising: (a) an oligonucleotide of any one of claims 1-11 and (b) a pharmaceutically acceptable carrier.

13. Use of an oligonucleotide of any one of claims 1-12 in the manufacture of a medicament for treating a bowel disease or treating or preventing a solid tumor, tumor invasion, or tumor metastasis.

14. Use of an antisense oligonucleotide or siRNA that inhibits the expression of fragile X mental retardation protein (FMRP) in the manufacture of a medicament for treating a bowel disease or treating or preventing a solid tumor, tumor invasion, or tumor metastasis.

15. The use of claim 14, wherein the antisense oligonucleotide or siRNA comprises a sequence selected from the group consisting of:
5′-CCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 1),
5′-CTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 2),
5′-TCCACCACCAGCTCCTCC-3′ (SEQ ID NO: 3),
5′-CTTCCACCACCAGCTCC-3′ (SEQ ID NO: 4),
5′-TCACCCTTTATCATCCTC-3′ (SEQ ID NO: 5),
5′-TCCACCACCAGCTCCTCCAT-3′ (SEQ ID NO: 6),
5′-ACTTCCACCACCAGCTCCTC-3′ (SEQ ID NO: 7),
5′-TTCCACCACCAGCTCCTCCA-3′ (SEQ ID NO: 8),
5′-ACTTCCACCACCAGCTCCT-3′ (SEQ ID NO: 9), and
5′-CTCACCCTTTATCATCCTCA-3′ (SEQ ID NO: 10), or a complement thereof.

16. The use of any one of claims 13-15, wherein the bowel disease is colorectal cancer or an inflammatory bowel disease; the inflammatory bowel disease is Crohn’s disease or ulcerative colitis; and/or the tumor is a colorectal cancer tumor.

17. The use of any one of claims 13-16, wherein the medicament is to be administered to a patient enterally or parenterally.

18. The use of claim 17, wherein the enteral administration is oral, sublingual, gastric, or rectal administration.

19. The use of claim 17, wherein the parenteral administration is intravenous, intratumoral, intrajejunal, intraileal, intracolonic, or intrarectal administration.

20. The use of any one of claims 13-19, wherein the medicament is for use in a human.