The Ala134Thr variant in TMEM176B exerts a beneficial role in colorectal cancer prognosis by increasing NLRP3 inflammasome activation

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Research Article

Keywords: TMEM176B, inflammasome, NLRP3, SNP, colorectal cancer

Posted Date: July 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1782224/v1

Abstract

Purpose

TMEM176B was recently described as a negative modulator of Nlrp3 inflammasome activation in mice. In the mouse model, the inhibition of TMEM176B leads to an increased anti-tumoral activity which is dependent on Nlrp3. Since we have recently shown that single nucleotide variants (SNPs) in inflammasome genes, including *NLRP3*, significantly affect colorectal cancer (CRC) prognosis, we proposed to investigate here the association between genetic variants in *TMEM176B* and CRC prognosis.

Methods

Considering that, up to now, no genetic study analyzing this gene in humans exists, we selected possible functional SNPs and genotyped them in a cohort of CRC patients submitted to surgery and followed up for more than 10 years. Genotype-guided assays were realized to evaluate the effect of the variant on NLRP3 inflammasome activation. Gene expression from The Cancer Genome Atlas (TCGA) cohort were analyzed to valid possible prognostic and predictive features.

Results

We identified the Ala134Thr variant (rs2072443) in TMEM176B as a protective factor for CRC prognosis. This SNP is associated with decreased gene expression and with an increased activation of NLRP3 inflammasome, at least in monocytes and dendritic cells. Furthermore, low *TMEM176B* expression is associated with higher overall survival.

Conclusion

Altogether these findings supported the role of TMEM176B in NLRP3 inflammasome biology and for the first time demonstrated the genetic association between rs2072443 and CRC in humans.

1. Introduction

Colorectal cancer (CRC) presents heterogeneous prognostic after surgery depending on several factors, such as age at diagnosis, tumor staging, lifestyle habits (smoke, alcohol, sedentarism, unhealthy diet), as well as molecular and immune tumor classification. Patient genetics background also contributes to the disease outcome and several genes related with immune response or epithelial homeostasis have been associated to CRC development and/or prognosis (De Mattia, 2020) [2].

We recently reported the significant association between two common single nucleotide polymorphisms (SNPs) in *NLRP1* and *NLRP3* genes, and a worse prognosis of CRC in patients who underwent surgery

(Cambui, 2020) [1]. These genes codify for ubiquitous innate immune receptors NLRP1 and NLRP3, which activate the production of the two pro-inflammatory cytokines IL-1ß and IL-18 through the activation of inflammasome complex and caspase-1. The associated SNPs, namely *NLRP1* rs11651270 (Val1164Met) and *NLRP3* rs35829419 (Gln705Lys), have been reported to be gain-of-function variants leading to an increase in inflammasome activation and cytokines release (Levandowski, 2013; Verma, 2012) [3–4]. These findings support the worst effect of an inflammatory microenvironment for the CRC development and outcome as demonstrated by other authors in animal models (largely reviewed in (Kolb, 2014) [5]. On the other side, it is well known that the constitutive inflammasome-mediated release of IL-18 by intestinal epithelial cells is important for the maintenance of intestinal homeostasis (Rathinam, 2018) [6]. Accordingly, we also reported that loss-of-function variants in *IL18* gene were associated with increased risk for CRC (Cambui, 2020) [2]. These findings lead us to hypothesize that the hyperactivation of inflammasome in immune cells, but not in epithelial cells, could play a detrimental role for CRC prognosis.

High stromal levels of the transmembrane protein 176B (TMEM176B), a cationic channel, were significantly associated with low overall survival of CRC patients [7]. In this same study, Segovia and colleagues demonstrated that the murine channel displays an inhibitory role on NLRP3 inflammasome-mediated IL-1ß production in dendritic cells, therefore affecting tumor infiltration and activation of CD8 + T cells (Segovia, 2019) [7]. Lack of *Tmem176b* induces increased activation of inflammasome, especially in the tumor-draining lymph nodes and not in the primary tumor, and restrains tumor growth in mice in an IL-1ß and caspase-1 dependent manner (Segovia, 2019) [7].

Considering the apparent discrepancy between inflammasome contribution in our association study and TMEM176B findings, as well as the recently described roles of this channel on NLRP3 inflammasome biology, we proposed to investigate here the association between common genetic variants in *TMEM176B* and CRC prognosis. We would like to emphasize that there is currently no genetic study analyzing this gene in humans.

2. Materials And Methods

2.1 CRC patients and healthy donors.

The genomic DNA of 187 adult patients with CRC was used for genotyping study. The CRC cohort was constituted of Brazilian adults who underwent surgery between 1994 and 2010 and were followed-up until 2019. Furthermore, CRC biopsies and peripheral leukocytes from 6 CRC patients who underwent surgery between 2020 and 2021 were used for gene expression analysis. Both cohorts were from the “Instituto de Tumores e Cuidados Paliativos" of the “Hospital Geral” (HG) and the “Clínica de Tratamento Multidisciplinar do Câncer” (ONCOMED) in Cuiabá (MT, Brazil). Main patients’ characteristics are resumed in **Table 1.**

Eighty-five (85) healthy donors (HD) (male/female = 37/48, age = 45.5 ± 13.4 years) were recruited at the Blood Bank Service of the “Oswaldo Cruz" Hospital (São Paulo, SP, Brazil) for genomic DNA extraction

and peripheral blood mononuclear cells (PBMC) isolation. All volunteers met the standards for donating blood of the Ministry of Health (BRASIL. Ministério da Saúde, 2014) [8].

2.2 DNA isolation and SNPs genotyping.

Genomic DNA was isolated by Salting out method (Miller, 1988) [9]. SNPs genotyping was performed using allele specific TaqMan® assays (Applied Biosystems, Thermo Fisher Scientific) and qPCR in a QuantStudio3 Real-Time PCR platform (Thermo Fisher Scientific). The QuantStudio 3.0 software was used for allelic discrimination.

2.3 PBMC isolation.

PBMC were isolated from donor blood using the Ficoll-Paque density gradient centrifugation according to manufacturer's instructions (GE Healthcare, Biosciences). Monocytes were separated from total PBMC by plastic adherence and cultivated at 4 x10^4 cells/mL in RPMI-10% FBS in 24-well culture plates. For some experiments, monocytes were cultured in the presence of 50 ng/mL GM-CSF (Peprotech) and 50 ng/mL IL-4 (Peprotech) for differentiation in monocyte-derived dendritic cells (MDDC), or M-CSF (Peprotech) for differentiation in monocyte-derived macrophages (MDM). CD4+ T and CD19+ cells were isolated from total PBMC by magnetic beads and negative selection (Miltenyi Biotec)), and cultured at 37°C in RPMI-10% FBS in 24-well culture plates.

2.4 PBMC stimulation and genotype-guided assay.

To activate the NLRP3 inflammasome, monocytes, MDM and MDDC isolated from 20 HD were stimulated with 1 µg/mL LPS (*E.coli,* strain: O111:B4, Sigma-Aldrich, Merck) for 4 or 24 hours. At the end of incubation 1 mM ATP (Sigma-Aldrich, Merck) was added for 15 minutes (Gattorno, 2007, Dos Reis, 2019, Souza, 2020) [10-12]. CD19+ cells were stimulated with 200 µg/mL ß-glucan (*A.faecalis*, Sigma-Aldrich, Merck) for 24 hours (Ali, 2017) [13]. CD4+ T cells were stimulated with 10 µg/mL anti-CD3 e 2 µg/mL anti-CD28 (Biolegend) for 72 hours (Arbore, 2016) [14]. Supernatants were collected for cytokines dosage. IL-1ß, IL-18 and TNF were measured in culture supernatants with the Human ELISA MAX™ Deluxe kits (Biolegend). Comparisons in cytokines release were made according to donors *TMEM176B* genotypes.

2.5 Public database analysis.

For phylogenetic analysis, human sequence containing the rs2072442 variant with 10 flanking base pairs was aligned with the same region taken from a whole genome sequencing across multiple species (primates, rats, mice) by the use of Ensembl public database (www.ensembl.org).

Gene Expression Profile Interactive Analysis (GEPIA) server (http://gepia.cancer-pku.cn/) was used to analyze the expression of *TMEM176B* and inflammasome genes in CRC and normal colon mucosa as well as in whole blood reported in “TCGA” and “GTEx” public databases, respectively. Basal genes expression is expressed as transcripts per million cells, TPM.

The “TCGA” database was also used to analyze the expression and the overall survival. Basal genes expression is expressed as fragments per kilobase of transcript per million fragments mapped, FPKM. For survival analysis was used the log-rank test, a.k.a the Mantel–Cox test, based on gene expression. The Cox proportional hazard ratio (HR) and the 95% confidence interval (CI) information were also included in the survival plots.

2.6 Data analysis.

The SNPs association study was realized by general linear model (GLM) multivariate using the package “SNPassoc” (version 1.9-2) and the R-project software (http://www.R-project.org, version 3.6.3). The Haploview software (Barrett, 2005) [15] was used to analyze the linkage disequilibrium among SNPs and to derive the haplotypes. Survival analysis was performed using Mantel-Cox test for SNPs and gene expression.

Cytokine concentrations were compared in groups defined by SNPs genotypes by the use of the Mann-Whitney test (two groups) or the Kruskal-Wallis (three groups) test followed by multi-comparisons post-test, respectively. For expression level comparison, a multi comparison t test was performed. The calculations were performed using the GraphPad Prism software (version 9.0).

Differences with p-value<0.05 were considered statistically significant.

2.7 Ethical statement.

All subjects gave informed consent, and the research protocols were approved by the corresponding institutional review boards on the conduct of research human subjects.

3. Results

3.1 SNPs Selection for Association Study

To the best of our knowledge, this is the first human association study with focus on the candidate gene *TMEM176B.* Using the “Ensembl” portal we identified 15 SNPs localized within the coding region (**Fig. 1a, Table 2**).

Taking into account the minor allele frequency (MAF > 10-20 %) in European and African population (from “Ensembl” database and 1000genome Project), the possible functional effect, and Linkage Disequilibrium data **(Fig. 1b),** we selected 4 SNPs out of 15: rs11546674 C>A (5’UTR), rs3173833 A>T (p.Ser94Cys), rs2072443 C>T (p.Ala134Thr), and rs2302480 G>A (3’UTR).

According to eQTL analysis (from “GTEx" Portal), rs2302480 and rs3173833 are associated with an increased *TMEM176B* expression, whereas rs2072443 and rs11546674 with a diminished gene expression **(Table 2**). A previous gene expression analysis followed by eQTL evaluation realized in a case/control cohort of multiple sclerosis reported that rs3173833 and rs2072443 were associated with

the expression of *TMEM176B* in the blood however any significant difference in SNPs distribution has been detected (Nickles, 2013) [16].

As no data exist about *TMEM176B* variants in the Brazilian population, we first analyzed the SNPs distribution in a representative cohort of healthy donors. The observed MAF did not significantly differ from the expected ones, calculated as a mean between European and African MAF (Fisher test $p > 0.05$). Moreover, genotypes distribution resulted in Hardy-Weinberg equilibrium ($p > 0.05$) **(Table 3).**

3.2 Protective role of *TMEM176B* rs2072443 variant in CRC prognosis

TMEM176B SNPs were genotyped in a cohort of 187 unrelated CRC patients.

Before performing the association study, a linear regression analysis was executed to identify confounder factors for each principal variable (lethality, relapse, survival). As a result, sex, age at diagnosis, TNM stage and CEA level before surgery were included as correction variables in the subsequent multivariate analysis.

The unique SNP that resulted significantly associated with CRC prognosis was the rs2072443 C>T (p.Ala134Thr) **(Table 4, Supplementary Table 1)**. Patients carrying this variant in a dominant model of inheritance for the minor T allele appear to be more protected against CRC-related death ($p_{adj} = 0.009$: $OR_{adj} = 0.19$) and less prone to tumor relapse after surgery ($p_{adj} = 0.026$: $OR_{adj} = 0.22$) than non-carriers. Accordingly, rs2072443 carriers showed increased survival rate ($p_{adj} = 0.036$).

When survival analysis was performed by Mantel-Cox test, again the rs2072443 SNP resulted significantly associated with increased survival rate after 120 months from surgery, according to a recessive model for the minor T allele ($p = 0.038$) **(Fig. 1c)**.

Haplotype's analysis did not report any significant results (data not shown).

Altogether these data demonstrated for the first time the significant and protective association of the variant rs2072443 in *TMEM176B* gene with CRC prognosis.

3.3 *TMEM176B* rs2072443 variant is associated to increased IL-1ß release

The rs2072443 SNP is located in exon 5 of *TMEM176B* gene (**Fig. 1a**) and lead to an amino acid change (p.Ala134Thr) which is considered to be "tolerated" or "probably damaging" according to the tool used for the prediction of effect on protein function (SIFT or PolyPhen) (from the "Ensembl" website). The substitution affects a highly conserved residue (**Fig. 2a**) localized in the third transmembrane domain of the channel, possibly affecting the helix conformation of the region and maybe the correct folding of the protein. Unfortunately, no resolved structure is available yet to infer a conformational change caused by the variant (from "UniProtKB/Swiss-Prot" database).

According to the “GTEx” portal *TMEM176B* appears to be expressed in colon as well as in whole blood **(Fig. 2b).** The eQTL analysis (from the “GTEx” portal) reports a significant lower expression level of *TMEM176B* gene both in whole blood and colon from individuals carrying the T/T genotype of rs2072443 SNP compared to non-carriers **(Fig. 2c-d)**, suggesting that the SNP negatively affects *TMEM176B*, at least at transcriptional level.

Analysis performed using data from the “TCGA” public database showed that high levels of *TMEM176B* expression are associated with a poor 5-year survival (p = 0.012) (**Fig. 2e**). Moreover, *TMEM176B* expression is incremented in disseminated CRC tumors (TNM III and IV) compared to localized tumors (TNM I and II) (p = 0.043) (**Fig. 2f).**

Therefore, either the progression and the severity of CRC appeared to be associated with an augmented TMEM176B expression, confirming the possible eQLT effect of rs2072443 and the previously published data by Segovia and colleagues (Segovia, 2019) [7].

To elucidate the meaning of *TMEM176B* association with CRC in the context of NLRP3 inflammasome activation **(Fig. 2g**), we then tried to characterize the effect of rs2072443 SNP on the complex.

TMEM176B was first described in the myeloid compartment specifically in murine dendritic cells (Condamine, 2010) [17]. However, the activation of NLRP3 inflammasome has been reported in myeloid as well as lymphoid cells in humans (Shelbi, 2020, Ali, 2017, Arbore, 2016) [18,13-14]. To depict the effect of the variant on NLRP3 inflammasome in distinct types of leukocytes, we performed a genotype-guided assay in monocytes, MDM and MDDC, CD4+ T and CD19+ lymphocytes from HD. The cells were stimulated with classical stimuli for NLRP3 inflammasome activation: LPS followed by ATP for monocytes, MDM and MDDC (Gattorno, 2007, Dos Reis, 2019, Souza, 2020) [10-12], ß-glucan for B lymphocytes (Ali, 2017) [13] polyclonal stimulation with anti (**α**)-CD3 and **α**-CD28 for CD4+ T lymphocytes (Arbore, 2016) [14]. Main findings are included in **Fig. 2** (complete results in **Supplementary Figure 1**).

Monocytes and MDM from HD carrying the rs2072443 variant displayed significantly increased IL-1ß release in response to LPS and ATP (**Fig. 2h-i**). It is worth highlighting that we were not able to detect significant amounts of IL-18 in stimulated monocytes supernatants and for MDM we observed reasonable release of that cytokine however without significant difference among genotypes.

No significant differences were observed for other conditions or cells (**Supplementary Figure 1**), suggesting a cell-specific effect of this SNP, possibly depending on the expression of *TMEM176B* or either on the function played in different leukocytes. As expected, rs2072443 did not affect the secretion of a caspase-1-independent cytokine, such as TNF-α **Supplementary Figure 1).**

As TMEM176B has been recently described as a negative regulator of NLRP3/caspase-1-dependent IL-1ß release in mice (Segovia, 2019) [7], our results fit well with the eQTL data in humans: the less *TMEM176B is* expressed, the less it can inhibit NLRP3 inflammasome and IL-1ß release.

4. Discussion

Currently, either a beneficial (Allen, 2010, Zaki, 2010, Dupaul-Chicoine, 2015) [19-21] and a detrimental (Huber, 2012, Wang, 2016) [22,23] role of NLRP3 inflammasome, IL-1ß and IL-18 has been reported in tumorigenesis of colitis-associated colorectal cancer model in mice. Several studies support the idea that the beneficial role of NLRP3 inflammasome in cancer is dependent on IL-18 release rather than IL-1ß (Karki, 2017) [24], possibly due to the anti-tumoral role of IL-18 in immune response (Mantovani, 2019) [25] and to the homeostatic effect of the cytokine on gut epithelium (Rathinam, 2018) [6]. However, it is important to consider that the IL-1ß effect in cancer is cell-type specific, being pro-tumorigenic on epithelial cells and anti-tumorigenic on myeloid cells (Dmitrieva-Posocco, 2019) [26]. Some type of cancer, such as breast cancer (Tulotta, 2021) [27], can be benefited by the treatment with anti-IL-1ß drugs.

Taking into account the heterogeneous response of CRC patients to tumor removal and the variable response to post-surgery therapy, the characterization of factors that could affect patient outcome is urgently needed.

Differently from tumorigenesis, CRC prognosis is scarcely addressed in animal models, and very little in humans. As far as we know, there are only three independent association studies demonstrating a role for inflammasome in CRC prognosis, including one recently published by our group. Two of these studies show a gain-of-function variant in *NLRP3* as a risk factor for a poor CRC prognosis (Ungerbäck, 2012, Cambui, 2020) [28, 2], and one study show that a higher NLRP3 expression is associated with poor overall survival (Wang, 2020) [29].

Segovia et al. [7] recently demonstrated the key role of the TMEM176B channel on the anti-tumoral check-points therapy (anti-CTLA4, anti-PDL1) in a CRC mouse model, linking the up-regulation of that molecule with the inhibition of NLRP3/caspase-1/IL-1ß pathway, which in turn is relevant for dendritic cells' activation and adaptive cytotoxic response.

Trying to depict the role of TMEM176B in CRC prognosis, we performed an association study with selected possibly functional SNPs in *TMEM176B* gene. We demonstrated a protective effect of the Ala134Thr variant in one of the transmembrane domains of TMEM176B (rs2072443) in CRC prognosis. Although we analyzed a limited number of SNPs in *TMEM176B*, it is important to emphasize that the expected frequency of the associated variant is quite common (35%) in the general population, possibly being an important factor for patients' response. According to public database GTEx, Ala134Thr variant leads to a diminished expression of *TMEM176B*, however the available data do not allow us to identify an eventual cell specific effect, as the analysis is by tissue and not in isolated cells. *TMEM176B* gene appears to be expressed in colon and in whole blood, therefore we can expect a SNP effect in both compartments. In colon, the minor expression of *TMEM176B* may lead to increased release of IL-18 which exerts a beneficial role onto the gut mucosa (Rathinam, 2018) [6]. In the immune compartment a reduced inhibition of inflammasome may result in a major propension to release IL-1ß and/or IL-18 depending on the leukocyte.

Our *in vitro* assays confirmed, at least in part, the effect of rs2072448 variant on NLRP3 inflammasome activation.

Further analyzes are required to better understand the effect of TMEM176B inhibition in tumor control.

Conclusions

TMEM176B has been recently discovered as a novel NLRP3 modulator and its chemical inhibitor BayK8644 has been shown to restore an efficient anti-tumoral response in mice [7]. Here we have demonstrated for the first time the protective effect of the rs2072443 variant in *TMEM176B* in CRC prognosis. No functional data are still available for the SNP, however, we have shown that rs2072443 lead to a rise in IL-1ß release in myeloid cells. Gene expression and inflammasome activation analysis suggest that the protective effect of *TMEM176B* SNP is mainly exerted in immune infiltrating cells rather than in CRC cells, therefore underlining the complexity of the tumoral microenvironment.

Declarations

Authors' contribution

R.A.G.C. and A.P. designed the study, analyzed the data, and wrote the manuscript and did the association study and genotype-guided assays. R.A.G.C., V.N.C.L. and F.P.F performed the genotyping assay. V.N.C.L., E.C.R. and D.S.L. realized the cell culture assays. G.F.E.S. and R.M.E. recruited the CRC patients and collected the clinical data.

Fundings: This study was supported by "Fundação de Suporte a Pesquisa do Estado de Sao Paulo" (FAPESP, grant number 19/06363-4). A.P. is a recipient of a Fellowship from "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq, grant number 302206/2019-1). R.A.G.C. is a recipient of a PhD Fellowship from "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES, grant number 88887.469122/2019-00). The other authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval: All the experiments involving human participants were approved by the Ethics Committee in Research In Human Beings (CEPSH) of the Institute of Biomedical Science/University of Sao Paulo.

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Conflict of interest: The author(s) declare that they have no conflict of interest.

Data availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

The authors would like to acknowledge to all the volunteers and the professional team from “Instituto de Tumores e Cuidados Paliativos" of the “Hospital Geral”, “Clínica de Tratamento Multidisciplinar do Câncer” (ONCOMED) and “Oswaldo Cruz” Hospital for the samples and clinical data. Authors also would like to acknowledge the Laboratory of Genetics and Molecular Biology, Institute of Medicine, Federal University of Mato Grosso (Brazil) headed by Dr. Bianca Borsatto Galera for the assistance with the samples handling and storage.

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Tables

Table 1 - Demographic and clinical data of Brazilian CRC cohort.

Characteristics	**Patients** (n)	M**edian** (interval)
Sex, Male/Female	103/84	-
Family history, Yes/No	91/89	-
IBD, Yes/No	13/164	-
Age at diagnosis, years	187	57 (20 - 85)
TNM staging: Initial (I-II)/ Advanced (III-IV)	94/88	-
Spreading to lymph nodes, Yes/No	75/99	-
CEA before surgery (CEA_0), mg/dL	156	101.99 (0.50 -7047.00)
Remission, Yes/No	104/68	-
Survival, months	122	80 (0 - 228)
Relapse, Yes/No	31/121	-
CEA at relapse (CEA_r), mg/dL	34	195.39 (0.53 - 4574.0)

Table 2 - List of SNPs in *TMEM176B coding region.*

SNPs in the *TMEM176B* coding region with a MAF > 0,1 according to the public database “Ensembl" (www.ensembl.org). Polymorphism identifiers (SNP ID), chromosomal position (Chr: bp), alleles, frequency of the minor allele (MAF) in the European (EUR) and African (AFR) populations are reported, as well as the consequence and the predicted effect on gene expression according to the GTEx website. Grey cells referred to amino acid changes.

SNP ID	Chr: bp	Alleles	EUR MAF	AFR MAF	Consequence	Predicted effect (GTEX)
rs1108577	7:150801331	A>T	0.32 (T)	0.22 (T)	5'UTR variant	not found
rs114949263	7:150801157	T>C	0.10 (C)	0.03 (C)	5'UTR variant	decreased expression (C)
rs13650	7:150800957	A>G	0.27 (G)	0.62 (G)	5'UTR variant	not found
rs55775933	7:150800839	A>G	0.26 (G)	0.11 (G)	5'UTR variant	decreased expression (G)
rs115946508	7:150800408	C>A	0.10 (A)	0.04 (A)	5'UTR variant	decreased expression (A)
rs11546674	7:150800332	C>A	0.52 (A)	0.47 (A)	5'UTR variant	decreased expression (A)
rs11769511	7:150800059	G>A	0.18 (A)	0.30 (A)	5'UTR variant	not found
rs62490424	7:150796662	C>T	0.16 (T)	0.03 (T)	5'UTR variant	increased expression (T)
rs11546671	7:150796407	C>A	0.03 (A)	0 (A)	p.Pro55Ser	not found
rs3173833	7:150793996	A>T	0.42 (T)	0.50 (T)	p.Ser94Cys	increased expression (T)
rs2072443	7:150793288	C>T	0.42 (T)	0.29 (T)	p.Ala134Thr	decreased expression (T)
rs17256042	7:150793150	G>A	0.06 (A)	0.01 (A)	p.Arg180Trp	not found
rs2302479	7:150791528	T>A	0.41 (A)	0.19 (A)	3'UTR variant	increased expression (A)
rs2302480	7:150791407	G/A	0.17 (A)	0.19 (A)	3'UTR variant	increased expression (A)
rs2302481	7:150791380	A/G	0.17 (G)	0.19 (G)	3'UTR variant	increased expression (G)

Table 3. Distribution of selected SNPs in healthy donors.

SNPs ID, variant alleles, expected and observed minor allele frequency (MAF) are reported as well as the results of MAF comparisons (p) and of the Hardy-Weinberg test (H-W p).

SNP ID	Alleles	Expected MAF	Observed MAF	p	H-W p
rs11546674	C>A	0.50 (A)	0.47 (A)	0.549	0.308
rs3173833	A>T	0.46 (T)	0.49 (T)	0.547	0.568
rs2072443	C>T	0.36 (T)	0.35 (T)	0.835	0.224
rs2302480	G>A	0.18 (A)	0.21 (A)	0.434	0.243

Table 4. Multivariate analysis results for the association study of *TMEM176B* rs2072443 and CRC prognosis after ablative surgery.

rs2072443 SNP distribution in CRC patients was analyzed according to lethality (yes/no), relapse (yes/no) and survival (in months) by multivariate analysis and general linear model (GLM). All analyses were adjusted for confounder variables (gender, age at diagnosis, TNM stage and CEA level before surgery). Differences in SNP distribution with $p_{adj} < 0.05$ are considered statistically significant.

Lethality	**Yes (n=48)** count (%)	**No (n=134)** count (%)	**p_{adj}**	**OR_{adj} (95% CI)**
C/C	12 (0.25)	11 (0.08)	0.009	0,19 (0.67-0.054)
C/T	25 (0.53)	68 (0.51)		
T/T	11 (0.22)	55 (0.41)		
Relapse	**Yes (n=30)** count (%)	**No (n=120)** count (%)	**p_{adj}**	**OR_{adj} (95% CI)**
C/C	8 (0.28)	11 (0.09)	0.026	0,26 (0.08 - 0.83)
C/T	10 (0.32)	66 (0.55)		
T/T	12 (0.40)	43 (0.36)		
Survival	**Individuals** (n)	**Mean ± SE** (months)	**p_{adj}**	
C/C	18	56.9 ± 7.9144	0.036	
C/T	55	88.1 ± 8.7561		
T/T	48	84.9 ± 9.0793		

n: number of individuals: count: genotype count; %: genotype percentage; 95% CI: 95% confidence interval. SE: standard error.

Figures

Figure 1

Gene exons and protein domains organization of TMEM176B and SNPs localized in the coding region.

a) For the *TMEM176B* gene (NM_014020, 1444 bp) the chromosomal location, the number of exons (1-7), the CDS (374-1187 bp, in gray), the 5 'and 3' UTR regions (in white) are indicated. For the protein (NP_001094782) the four transmembrane protein domains (T) in the sequence of 270 amino acids are indicated. SNPs in the *TMEM176B* coding region are indicated (from the public database “Ensembl”). **b)** Linkage Disequilibrium (LD) plot for *TMEM176B* SNPs. LD = 1 is indicated by haploblocks. The color scheme shows in white LOD < 2 and D’ < 1, in shades of pink/red: LOD ≥ 2 and D’ < 1, bright red: LOD ≥ 2 and D’ = 1. **c)** Survival curve based on *TMEM176B rs2072443* patients’ genotypes. The Mantel-Cox test was used for analysis.

Figure 2

Analysis of rs2072443 (Ala134Thr) possible functional effect

a) Multi-species alignment of the nucleotidic sequence of rs2072443 C>T SNP and 10 bp flanking region in humans, primates, rats and mice. Polymorphic T allele and corresponding amino acid (Ala) are indicated in red. The correspondent codon is evidenced in yellow throughout the species. **b**) Tissue specific gene expression profile of TMEM176B and main NLRP3 inflammasome genes. Data are from the “GTEx” portal and is expressed as a transcript per million of cells (TPM). **c-d)** Expression level of *TMEM176B* in whole blood (C) and colon (D) of healthy individuals according to rs2072443 genotype. Data are from the “GTEx” portal and is expressed as a transcript per million of cells (TPM). Median and upper-lower intervals are indicated, as well as the number of individuals in each group. **e)** Survival curve based on expression level of *TMEM176B* in CRC tissue. The Mantel-Cox test was used for analysis. **f)** Expression level of TMEM176B in localized (TNM I-II) and disseminated (TNM III-IV) CRC tumor. Data are from the “TCGA” portal and is expressed as fragments per kilobase of transcript per million fragments mapped (FPKM). T test was used to compare the expression level. * : p< 0.05. **g)** Graphical representation of the supposed link between TMEM176B and NLRP3 inflammasome (from Segovia, 2019 [7]). **h-i)** IL-1ß release in monocytes (H) and monocyte-derived macrophages (MDM) (I) stimulated with 1 µg/mL LPS for 4 hours or 24 hours respectively, and then with 1 mM ATP for 15 minutes. One-Way Anova test was used to compare cytokine release among the three genotypes. * : p< 0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementarymaterialCRCTMEM.pdf
- SupplementaryFIle2.tiff