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Invited Review

The molecular background of mucinous carcinoma beyond MUC2

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Abstract

The increasing interest of the oncology community in tumour classification and prediction of outcome to targeted therapies has put emphasis on an improved identification of tumour types. Colorectal mucinous adenocarcinoma (MC) is a subtype that is characterized by the presence of abundant extracellular mucin that comprises at least 50% of the tumour volume and is found in 10–15% of colorectal cancer patients. MC development is poorly understood, however, the distinct clinical and pathological presentation of MC suggests a deviant development and molecular background. In this review we identify common molecular and genetic alterations in colorectal MC. MC is characterized by a high rate of MUC2 expression. Mutation rates in the therapeutically important RAS/RAF/MAPK and PI3K/AKT pathways are significantly higher in MC compared with non-mucinous adenocarcinoma. Furthermore, mucinous adenocarcinoma shows higher rates of microsatellite instability and is more frequently of the CpG island methylator phenotype. Although the majority of MCs arise from the large intestine, this subtype also develops in other organs, such as the stomach, pancreas, biliary tract, ovary, breast and lung. We compared findings from colorectal MC with tumour characteristics of MCs from other organs. In these organs, MCs show different mutation rates in the RAS/RAF/MAPK and PI3K/AKT pathways as well, but a common mucinous pathway cannot be identified. Identification of conditions and molecular aberrations that are associated with MC generates insight into the aetiology of this subtype and improves understanding of resistance to therapies.

Keywords: mucinous carcinoma; colorectal carcinoma; molecular pathology; phenotype; genotype

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Introduction

Rapid development of individualized therapy for cancer patients has led to an increased attention for tumour subtypes. The search for therapeutically relevant pathways has been ongoing and molecular classification of cancer has become an important component in clinical decision making. Identification of the molecular background of tumours is one of the key challenges in cancer research, as it improves understanding of tumour development and may predict responsiveness to therapies.

Annually, approximately 1.2 million patients develop colorectal carcinoma (CRC) worldwide and the non-mucinous adenocarcinoma (NMC) forms the vast majority of these patients [1]. However, in 10–15% of cases, mucinous adenocarcinoma (MC) is diagnosed. MC is a subtype that is characterized by the presence of abundant extracellular mucin comprising at least 50% of the tumour volume [2]. Compared with NMC, MC is more frequently found in the proximal colon and has a higher stage at presentation [3,4]. Moreover, MCs have a distinct metastatic pattern and are less responsive to palliative chemotherapy [5–8]. The relatively rare occurrence of colorectal MC renders it a less well-studied entity and MC development is not well understood. Nevertheless, the distinct clinical and pathological presentation suggests a deviant development and molecular background.

Although the majority of MCs arises from the gastrointestinal tract, they are also found in various other organs. Overexpression of MUC2 is a common finding in MCs, but it does not explain the distinct biology of these tumours [9]. Identification of conditions and molecular aberrations that are associated with MC may generate insight into the pathways leading to the development of this subtype and improves understanding of resistance to therapies. In this review, we identify common molecular and epigenetic alterations in colorectal MC and compare findings with MCs from other organs.

Methods

Review of literature

The literature was searched with a Boolean search term combination until December 2013, using PubMed and EMBASE. Titles and abstracts were evaluated to identify relevant studies, which were assessed in full text. Reference lists of retrieved studies were explored for further relevant publications. Only studies that contained data on molecular or genetic characteristics and that compared MC and NMC (at least five patients per subtype) were selected. Studies that did not adhere to the definition of MC as reported in the guidelines of the World Health Organization (WHO) were excluded from the analyses [2]. Overlap between study populations was assessed, and in case of overlap, only the most recent data were used for analysis. Differences between categorical outcomes were calculated using the risk ratio (RR) and corresponding 95% confidence interval (CI). Heterogeneity was assessed by means of the I^2 statistic. The existence of publication bias in the meta-analyses was assessed using funnel plots.

The Cancer Genome Atlas project

The Cancer Genome Atlas (TCGA) project was established to profile genomic changes in different cancer types. Data on 32 somatic recurrently mutated genes in CRC were published in 2012 by the TCGA group, and data from this study were available online [10]. Data on somatic mutations that were involved in the RAS/RAF/MAPK and PI3K/AKT pathways were downloaded on 22 December 2013. We only selected samples that were designated as either MC or NMC. A total of 28 MCs and 160 NMCs were identified from the TCGA dataset of this publication. Fisher's exact test was used for comparing mutation rates between MC and NMC. Statistical analyses were two-sided and p values <0.05 were considered significant.

Molecular determinants in MC

In CRC development, acquisition of mutations leads to abnormal cell division and uncontrolled cell growth. There are several well-recognized molecular pathways in CRC development [11]. Chromosomal instability (CIN), microsatellite instability (MSI) and hypermethylation of CpG islands are genetic instability pathways involved in carcinogenesis. Mutations in targets of the RAS/RAF/MAPK and PI3K/AKT pathways are common findings in CRC. These important drivers of cancer development are of prognostic and predictive importance and are being explored for targeted therapies.

MUC2

Secreted gel-forming mucins are epithelial glycoproteins that play a role in physiological processes of the gastrointestinal tract. They are encoded by the *MUC2*, *MUC5AC*, *MUC5B* and *MUC6* genes on chromosome 11p15.5 [12]. *MUC2* is of particular interest with regard to its role in CRC as the expression of MUC2 is generally decreased in CRC [13]. Interestingly, an increase of MUC2 has been observed in MCs, which also explains the mucinous appearance of these tumours [14–16]. A meta-analysis by Li *et al* demonstrated a higher rate of MUC2 positivity in MC compared with NMC (RR 2.10, 95% CI 1.30–3.40) [17]. Overexpression of MUC2 was one of the first molecular aberrations that distinguished MC from NMC and is related to the low methylation status of the promoter of the *MUC2* gene in MC [18].

Microsatellite instability

Loss of mismatch repair (MMR) mechanisms causes MSI, which is the hallmark of Lynch syndrome-associated tumours. Lynch syndrome (previously known as hereditary non-polyposis colorectal cancer) is an autosomal dominantly inherited cancer predisposition syndrome, caused by germline mutations in MMR genes. MC accounts for 22–40% of Lynch syndrome-associated CRCs [19]. MSI is also found in approximately 12% of CRC patients who do not suffer from a hereditary predisposition [20]. The prevalence of MC has been reported to be 11–77% in

Table 1. Reports on MC among patients with sporadic colorectal cancer with MSI

Study	Year	Patients with MSI in study	% MC
Kim [21]	1994	18	33.3
Bocker [22]	1996	11	36.4
Gafà [23]	2000	44	36.4
Young [24]	2001	42	42.9
Hawkins [25]	2002	43*	41.9
Shia [26]	2003	35*	11.4
Sarli [27]	2004	22	77.3
Mori [28]	2004	14	28.6
Chang [29]	2006	19*	31.6
Meng [30]	2007	12*	50.0
Ashktorab [31]	2008	6	33.3
Kim [32]	2010	135*	15.6
Kakar [33]	2012	14	50.0
Day [34]	2013	134*	43.3
Total		**549**	**34.1**[†]

*Bethesda panel was used for determination of MSI status.
[†]Overall weighted average according to the number of patients in each study.

sporadic MSI CRC patients (weighted average of 34%, Table 1) [21–34]. Studies that directly compared sporadic MSI and Lynch syndrome-associated CRCs found a higher rate of MC in sporadic MSI CRCs than in Lynch syndrome-associated CRCs [24,35]. A better survival in MC patients has been reported for tumours exhibiting MSI compared with microsatellite stable tumours [36–38]. However, comparison of MSI rates between studies is difficult, as a wide variety of markers for determining MSI status is used.

MSI can also occur through hypermethylation of the hMLH1 promoter region, which is seen in CRCs that display the CpG island methylator phenotype (CIMP). CIMP is characterized by hypermethylation of CpG islands in the promoter region of genes involved in carcinogenesis, leading to epigenetic silencing [25,39–41]. Studies found 36–41% of MCs to be CIMP positive, compared with only 12–18% in NMC (supplementary material, Figure S1) [25,33,42–44]. Tanaka *et al* demonstrated that MCs more frequently have MSI or CIMP or BRAF mutations than NMCs (54% versus 28%) and as the various characteristics are correlated, this is indicative for MC arising from an alternative oncogenic pathway [40]. The sequence of these mechanisms is not yet completely understood.

KRAS

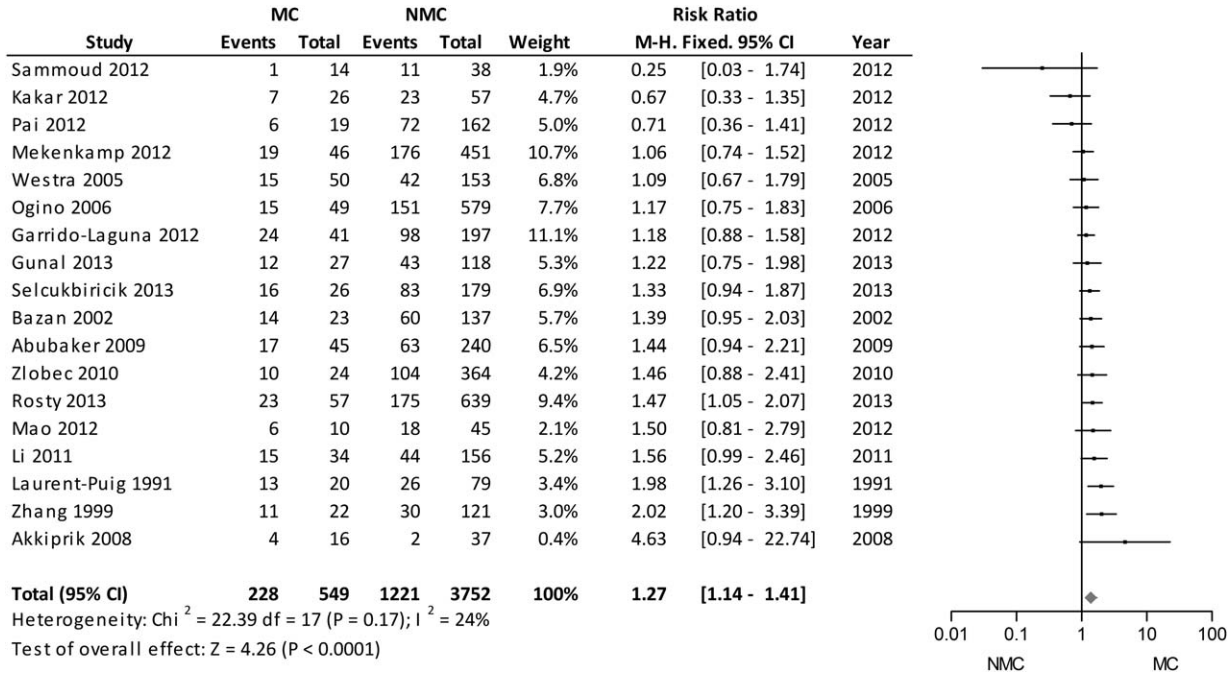
Mutations in *KRAS* lead to an epidermal growth factor receptor (EGFR)-independent disturbance of the RAS/RAF/MAPK pathway, that regulates cell proliferation and survival and is a prognostic factor in CRC [45,46]. Conflicting results have been reported in the literature regarding the incidence of *KRAS* mutations in MC. Rates of mutant *KRAS* are varying between 7–65% in MC versus 5–50% in NMC. Often, results were not statistically significant, possibly due to lack of power. Eighteen studies were included in an analysis on *KRAS* status in MC and NMC and *KRAS* mutations were found in MC more frequently (RR 1.27, 95% CI 1.14–1.41; Figure 1) [6,33,47–62].

BRAF

Mutated *BRAF* is another molecular aberration that is more frequently found in MC patients. *BRAF* is the downstream effector of *KRAS* and is also involved in the RAS/RAF/MAPK pathway. In various studies, mutational *BRAF* was found in 0–46% of MC patients, whereas 6–25% of NMC tumours displayed mutated *BRAF* (RR 2.04, 95% CI 1.67–2.51; Figure 2) [6,33,42,48,50,56–59,63]. *BRAF* mutations lead to constitutive activation of the RAS/RAF/MAPK signalling pathway [64]. A hotspot for *BRAF* mutations involves replacement of a single amino acid, V600, located within the kinase domain and accounts for 80% of *BRAF* mutations in CRC [65]. *BRAF* mutations are highly correlated with CIMP, with approximately 60–80% of CIMP tumours having *BRAF* mutations [40,50,66,67]. *BRAF* mutations are also frequently found in sporadic MSI CRC but not in Lynch syndrome-associated CRC [66–69].

PIK3CA

Activating mutations in *PIK3CA* occur in approximately 13% of CRCs (Figure 3). *PIK3CA* encodes a catalytic subunit of PI3K and is a positive regulator of the PI3K/AKT pathway, which is involved in cell growth, survival, proliferation and motility [70]. The PI3K pathway is normally inhibited by tumour suppressor gene *PTEN*. *PIK3CA* is more commonly mutated in MC (9–50%) than in NMC (7–12%) and a RR of 1.79 (95% CI 1.46–2.19) was found for MC in an analysis on mutational *PIK3CA* status [34,58,59,71–74]. Also, *PIK3CA* mutations occur more frequently in tumours that are localized in the proximal colon, as are MCs [3,4,34,73]. *PIK3CA* mutations are commonly found in combination with *KRAS* mutations and are associated with high levels of CIMP, which are both linked to MC [51,72,73]. An association between *PIK3CA* mutation and MSI has not been demonstrated [72]. In the literature,



Study	MC Events	MC Total	NMC Events	NMC Total	Weight	Risk Ratio M-H. Fixed. 95% CI	Year
Sammoud 2012	1	14	11	38	1.9%	0.25 [0.03 - 1.74]	2012
Kakar 2012	7	26	23	57	4.7%	0.67 [0.33 - 1.35]	2012
Pai 2012	6	19	72	162	5.0%	0.71 [0.36 - 1.41]	2012
Mekenkamp 2012	19	46	176	451	10.7%	1.06 [0.74 - 1.52]	2012
Westra 2005	15	50	42	153	6.8%	1.09 [0.67 - 1.79]	2005
Ogino 2006	15	49	151	579	7.7%	1.17 [0.75 - 1.83]	2006
Garrido-Laguna 2012	24	41	98	197	11.1%	1.18 [0.88 - 1.58]	2012
Gunal 2013	12	27	43	118	5.3%	1.22 [0.75 - 1.98]	2013
Selcukbiricik 2013	16	26	83	179	6.9%	1.33 [0.94 - 1.87]	2013
Bazan 2002	14	23	60	137	5.7%	1.39 [0.95 - 2.03]	2002
Abubaker 2009	17	45	63	240	6.5%	1.44 [0.94 - 2.21]	2009
Zlobec 2010	10	24	104	364	4.2%	1.46 [0.88 - 2.41]	2010
Rosty 2013	23	57	175	639	9.4%	1.47 [1.05 - 2.07]	2013
Mao 2012	6	10	18	45	2.1%	1.50 [0.81 - 2.79]	2012
Li 2011	15	34	44	156	5.2%	1.56 [0.99 - 2.46]	2011
Laurent-Puig 1991	13	20	26	79	3.4%	1.98 [1.26 - 3.10]	1991
Zhang 1999	11	22	30	121	3.0%	2.02 [1.20 - 3.39]	1999
Akkiprik 2008	4	16	2	37	0.4%	4.63 [0.94 - 22.74]	2008
Total (95% CI)	**228**	**549**	**1221**	**3752**	**100%**	**1.27 [1.14 - 1.41]**	

Heterogeneity: Chi ^{2} = 22.39 df = 17 (P = 0.17); I ^{2} = 24%

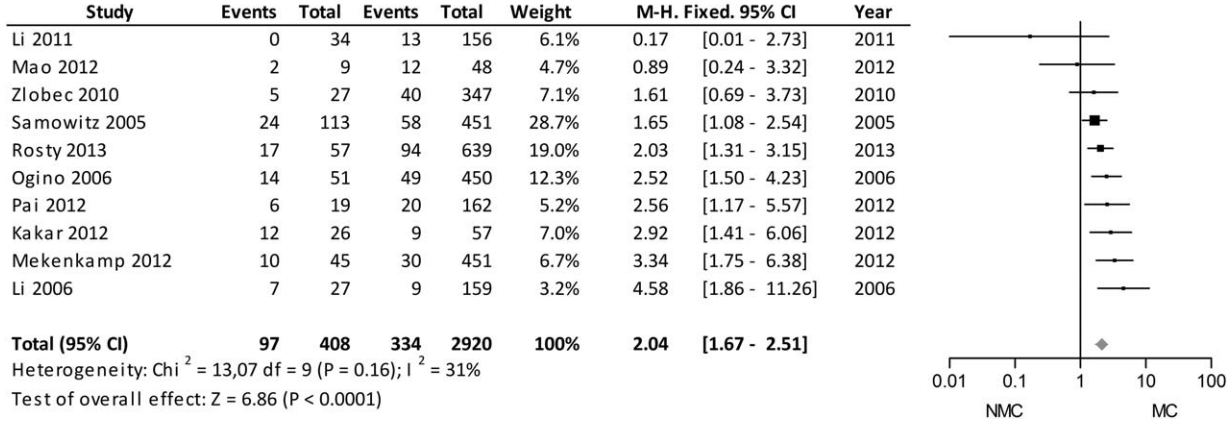
Test of overall effect: Z = 4.26 (P < 0.0001)

Figure 1. Relative risk for *KRAS* mutation in studies comparing colorectal MC and NMC.

conflicting results have been published regarding *PTEN*. A study by Day *et al* that analysed mutational status of *PTEN* found a higher frequency of *PTEN* mutations in MC (10% in MC versus 5% in NMC); however, studies that analysed cytoplasmic expression of PTEN did not always find a difference between MC and NMC [34,58,74,75].

TCGA

Besides findings from the literature, also unpublished data collected by TCGA offers possibilities to compare mutation rates in CRC. In 188 CRCs (28 MC and 160 NMC), the mutational status of genes involved in the RAS/RAF/MAPK and PI3K/AKT pathway was assessed (Figure 4). Also data on MSI were available. In concordance with the literature, MCs more often displayed MSI and a higher rate of *BRAF* and *PIK3CA* mutations was found in MC. Mutation rates for other genes were not significantly different. Inclusion of TCGA data into the analyses on mutational status did not significantly alter risk ratios for *BRAF* (RR 2.24, 95% CI 1.84–2.72), *KRAS* (RR 1.26, 95% CI 1.13–1.40) and *PIK3CA* (RR 1.82,



Study	MC Events	MC Total	NMC Events	NMC Total	Weight	Risk Ratio M-H. Fixed. 95% CI	Year
Li 2011	0	34	13	156	6.1%	0.17 [0.01 - 2.73]	2011
Mao 2012	2	9	12	48	4.7%	0.89 [0.24 - 3.32]	2012
Zlobec 2010	5	27	40	347	7.1%	1.61 [0.69 - 3.73]	2010
Samowitz 2005	24	113	58	451	28.7%	1.65 [1.08 - 2.54]	2005
Rosty 2013	17	57	94	639	19.0%	2.03 [1.31 - 3.15]	2013
Ogino 2006	14	51	49	450	12.3%	2.52 [1.50 - 4.23]	2006
Pai 2012	6	19	20	162	5.2%	2.56 [1.17 - 5.57]	2012
Kakar 2012	12	26	9	57	7.0%	2.92 [1.41 - 6.06]	2012
Mekenkamp 2012	10	45	30	451	6.7%	3.34 [1.75 - 6.38]	2012
Li 2006	7	27	9	159	3.2%	4.58 [1.86 - 11.26]	2006
Total (95% CI)	**97**	**408**	**334**	**2920**	**100%**	**2.04 [1.67 - 2.51]**	

Heterogeneity: Chi ^{2} = 13,07 df = 9 (P = 0.16); I ^{2} = 31%

Test of overall effect: Z = 6.86 (P < 0.0001)

Figure 2. Relative risk for *BRAF* mutation in studies comparing colorectal MC and NMC.

Study	MC Events	MC Total	NMC Events	NMC Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Year
Abubaker 2008	8	58	43	360	10.5%	1.15 [0.57 - 2.33]	2008
Li 2011	5	34	20	166	6.0%	1.22 [0.49 - 3.03]	2011
Mao 2012	1	11	4	56	1.2%	1.27 [0.16 - 10.33]	2012
Day 2013	33	210	75	725	29.8%	1.52 [1.04 - 2.22]	2013
Nosho 2008	44	233	36	361	24.9%	1.89 [1.26 - 2.85]	2008
Rosty 2013	50	236	43	445	26.3%	2.19 [1.51 - 3.19]	2013
Voutsina 2013	5	10	6	73	1.3%	6.08 [2.27 - 16.30]	2013
Total (95% CI)	**146**	**792**	**227**	**2186**	**100%**	**1.79 [1.46 - 2.19]**	

Heterogeneity: Chi ^{2} = 10.11, df = 6 (P = 0.12); I ^{2} = 41%

Test of overall effect: Z = 5.68 (P < 0.0001)

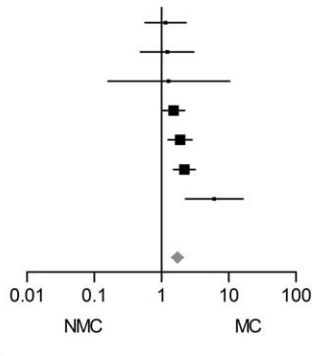


Figure 3. Relative risk for *PIK3CA* mutation in studies comparing colorectal MC and NMC.

95% CI 1.50–2.20). Mutations in *ERBB2* (which encodes HER-2) are considered uncommon in CRC and were found in only 7.1% and 6.3% of MC and NMC samples, respectively. In conclusion, data from TCGA confirmed differences in mutation rates between MC and NMC of several genes that were also reported in the literature.

Mucinous colorectal pathway

Findings from the literature and TCGA suggest that MC and NMC differ on a molecular basis (Figure 5). An increased rate of mutations is seen in MC in the RAS/RAF/MAPK and PI3K/AKT pathways. *KRAS*, *BRAF* and *PIK3CA* are more frequently mutated in MC compared with NMC, leading to constitutive activation of these pathways. No differences in expression of the cell surface receptors EGFR or HER-2, that are upstream of these pathways, have been reported between MC and NMC in the literature.

Although MSI, CIMP and activation of the RAS/RAF/MAPK and PI3K/AKT pathways are distinctive features of MC, the relationship between these characteristics and mucin production has not yet been elucidated. There is no data on a molecular link between MSI or CIMP and overexpression of MUC2. However, various *in vitro* studies demonstrated that both the RAS/RAF/MAPK and PI3K/AKT pathway are involved in MUC2 upregulation in colon cancer cell lines and indicated that MUC2 production can be inhibited by a MEK inhibitor [76–79]. In another cell line, however, upregulation of MUC2 was considered independent of MAP kinase [80]. Recently, Walsh *et al* reported data on 722 CRC patients, which supported the association between overexpression of MUC2 and activation of the RAS/RAF/MAPK pathway via *BRAF* and *KRAS* mutations [81]. They also found that MUC2 overexpression was associated with a deficient MMR system and CIMP. Especially the latter is surprising, as it indicates an increase in protein expression in an environment in which excessive silencing of gene promoters is present. These findings strongly suggest that overexpression of MUC2 in MCs is related to other molecular aberrations, but further evaluation is needed.

Compare and contrast

Besides the colorectal variant, MC is also found in tumours originating from other organs. MC has been described in patients suffering from carcinoma of the oesophagus, stomach, small intestine, pancreas, biliary tract, gall bladder, ovary, endometrium, urinary bladder, breast and lung. It is unknown whether MCs

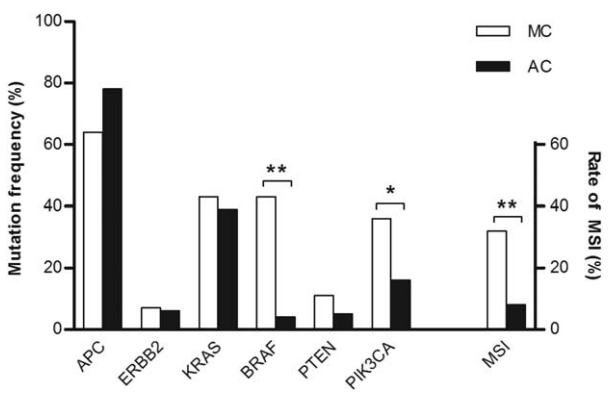


Figure 4. Rates of mutations and microsatellite instability in colorectal carcinoma: 28 MC and 160 NMC samples from the TCGA project. MSI testing was performed for 159 NMC samples; *$p < 0.05$, **$p < 0.01$.

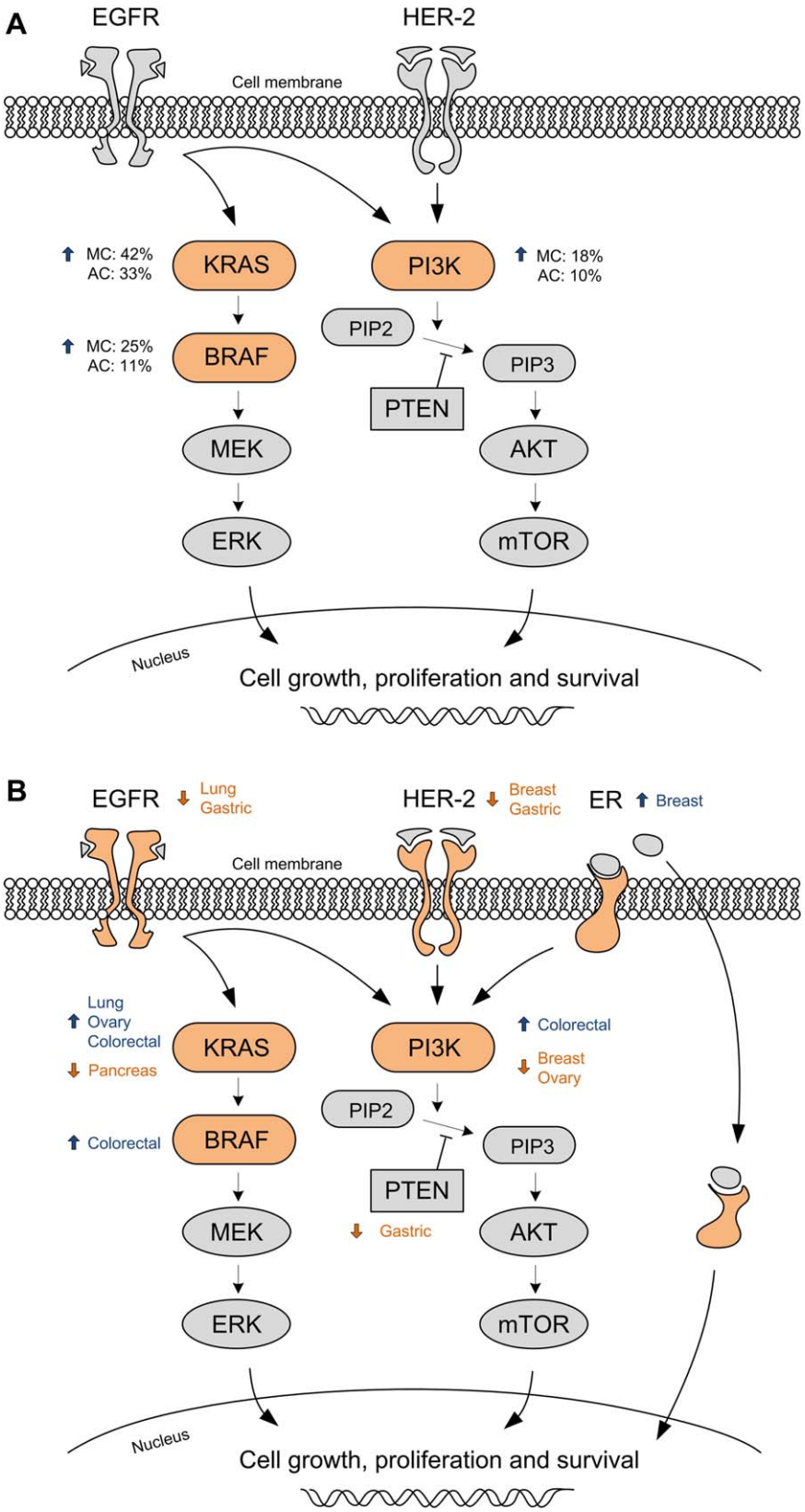


Figure 5. EGFR, HER-2 and ER with downstream the RAS/RAF/MAPK and PI3K/AKT pathway. (A) Mutation rates of *KRAS*, *BRAF* and *PIK3CA* are different between MC and NMC in colorectal cancer. (B) An increase or decrease in mutation or expression rates of components of the RAS/RAF/MAPK and PI3K/AKT pathway has been observed in MC when compared with NMC in different tumour types.

from different organs share common molecular characteristics. Hanski *et al* previously demonstrated that overexpression of the MUC2 gene was found in MCs from different organs [9]. The rare occurrence of MC in most organs is reflected by the limited number of studies regarding this subtype. In this section, MCs from variant organs are described, dependent on availability in the literature.

Mucinous gastric carcinoma

MC is one of the five main subtypes in the WHO classification system of gastric adenocarcinomas and comprises approximately 2–5% of all gastric cancers [28,82–84]. As most studies use the Laurén classification system, which divides gastric carcinoma in an intestinal and diffuse subtype, there is little data on gastric MC. Identical to colorectal MC, gastric adenocarcinoma is designated mucinous if more than 50% of the tumour consists of extracellular mucin [2]. Gastric MCs are more often diagnosed at a more advanced stage of disease than NMC, resulting in a poorer outcome [82,83,85].

Similar to colorectal MC, gastric MC is also associated with MUC2 overexpression [86,87]. Also, a higher rate of MSI is found in MC when compared with NMC (average of 14% versus 11%, RR 1.51, 95% CI 1.03–2.21; supplementary material, Figure S2) [86,88–91]. Similar to CRC, MSI has been associated with a better prognosis in gastric carcinoma [92,93]. HER-2 overexpression and *ERBB2* gene amplification are less common in MC than in NMC (1% versus 6%) [86,94]. A higher rate of 18qLOH, which is associated with adverse outcome, has been reported for gastric MC compared with NMC (52% versus 21%) [89]. Expression of PTEN seems to be less altered in gastric MC, compared with NMC; Kang *et al* found that 27% of NMCs displayed loss of PTEN whereas none of the MCs did [95]. Gastric MC is associated with lower rates of EGFR overexpression compared with NMC (5–11% versus 26–31%) [86,94,96]. Conversely, one small study by Liu *et al* found an *EGFR* mutation in two of the seven MCs [97]. Additionally, this study found no *KRAS* mutations in MC, while 12% of NMCs had a *KRAS* mutation.

Mucinous noncystic pancreas carcinoma

Mucinous noncystic carcinoma of the pancreas is a variant of ductal adenocarcinoma and is usually referred to as colloid carcinoma. In pancreatic colloid carcinoma, mucin accounts for more than 50% of the tumour [2]. It is considered an uncommon subtype and arises almost exclusively from the intraductal papillary mucinous neoplasm (IPMN). The rare occurrence is a limiting factor on knowledge of the molecular background of colloid carcinoma, but Adsay *et al* demonstrated a low mutational rate of *KRAS* (25%) in a small colloid carcinoma cohort, whereas *KRAS* is mutated in >90% of ductal adenocarcinomas [98,99]. As in colorectal MC, colloid carcinoma of the pancreas is associated with a high expression frequency of MUC2 compared with ductal adenocarcinomas [98,100]. However, in contrast with colorectal MC, MSI is not a common finding in colloid carcinoma of the pancreas. Lüttges *et al* found only one case of MSI among 12 colloid carcinomas [101].

Mucinous carcinoma of the gall bladder and extrahepatic bile ducts

MCs of the gall bladder and biliary ducts contain more than 50% extracellular mucin by definition of the WHO classification system [2]. In a population-based study on biliary tract cancers, MC was found in 5% of cases [102]. This study by Rashid *et al* also found a higher rate of MSI in MCs (33%) from the biliary tract, compared with NMCs (2%). A recent study by Dursun *et al* on 606 gall bladder carcinomas reported MC in 2.5% cases [103]. MUC2 expression, which is typically negative in NMC of the gall bladder, was positive in 86% of MCs. However, none of the MCs displayed MSI in this study.

Mucinous ovarian carcinoma

NMC of the ovary forms the majority of ovarian carcinomas and mainly consists of serous, clear cell and endometrioid carcinomas. MC is diagnosed in approximately 11–14% of ovarian carcinomas [104,105]. MC is more frequently found in an early stage of disease and is associated with a better survival than NMC [106–108]. Compared with CRC, the ovarian variant of MC is an ill-defined entity and is usually classified as MC when the tumour has an 'intestinal' or 'cervical gland-like' phenotype. Unlike in the colon, ovarian carcinoma is labelled mucinous when either intracellular or extracellular mucin is present, without requiring any strict quantification of the mucin component [109]. Practically, this means that the group of ovarian MC comprises those phenotypes that are defined as both NMC and MC in the colon. In the literature, presence of either intracellular or extracellular mucin is generally neither mentioned nor quantified.

In ovarian cancer, *KRAS* is more frequently mutated in MC (10–71%), than in NMC (2–25%, supplementary material, Figure S3) [110–119]. *BRAF* mutations are rare in ovarian carcinoma, with only

0–9% of MC and 0–4% of NMC showing this mutation [112,117,118]. There seems to be no significant role for MSI in the mucinous differentiation, with MSI in 0–55% of MC and in 2–62% of NMC (supplementary material, Figure S4) [120–124]. For *PIK3CA* and *PTEN*, literature is limited. Campbell *et al* reported that 8% of NMCs exhibited a *PIK3CA* mutation, whereas none of the MCs did [125]. *PTEN* mutations were found in up to 10% of ovarian carcinomas, but this was not different between histological subtypes [126,127]. *ERBB2* amplification does occur in ovarian carcinoma, but no obvious differences between MC (28%) and NMC (19%) have been found [116]. CIMP has been examined to a limited extent in ovarian carcinoma.

The interpretation of data concerning ovarian MC is further complicated by the fact that a considerable part of MC consists of metastases from primary tumours originating elsewhere in the body, mainly from the gastrointestinal tract [128–131]. Because differentiation between a primary MC and metastasis is difficult, it is possible that a proportion of carcinomas that are considered ovarian MC are in fact metastatic CRC. This might impede interpretation of the reported data, but it could also explain the high frequency of *KRAS* mutations in MC.

Mucinous lung carcinoma

Invasive mucinous adenocarcinoma (IMA) of the lung (formerly mucinous bronchioalveolar carcinoma) was separated from the non-mucinous subtype in the new international multidisciplinary classification system based on major clinical, pathological and genetic differences between both the subtypes [132]. IMA, however, is not the pulmonary equivalent of MC from the gastrointestinal tract, as mucin is found intracytoplasmic in this tumour. The colloid carcinoma, which is characterized by abundant extracellular mucin, shows more resemblance with colorectal MC. Pulmonary colloid carcinoma is a rare subtype (found in less than 0.5% of lung carcinomas) and is often found as a mixture with other NMC subtypes [133]. *KRAS* and *EGFR* mutations are the two most frequently mutated proto-oncogenes in adenocarcinoma of the lung, whereas *BRAF* mutations and MSI are rare in lung carcinoma [134,135]. The pathogenic mechanisms behind colloid carcinoma are largely unknown, but MUC2 is found to be strongly expressed [133]. Moreover, a study by Liu *et al* found a higher rate of *KRAS* mutations and a lower rate of *EGFR* mutations in colloid tumours when compared with other subtypes [136]. As EGFR tyrosine kinase inhibitors are of particular interest for lung cancer treatment, more insight into the molecular background of subtypes could improve targeting therapy.

Mucinous breast carcinoma

According to the WHO classification system of breast carcinomas, MC of the breast is found in 7% of breast cancers and consists of clusters of tumour cells floating in pools of extracellular mucin [109]. In the literature, a pure and mixed variant of MC have been distinguished. Pure MC of the breast consists exclusively of MC and represents approximately 2% of all breast cancers [109]. The mixed variant of MC shows an admixture with another component (usually infiltrating ductal carcinoma, IDC) [109]. Compared with IDC, pure MC is a less-aggressive subtype that is rarely associated with lymph node metastases [137–140].

Comparison at the molecular level shows that MC is transcriptionally distinct from IDC [141,142]. MC is more homogenous at the genetic level and shows less genetic instability than most other types of breast cancer [141,143,144]. MC of the breast is associated with higher rates of MUC2 expression than IDC [98,145]. MC also has a higher rate of oestrogen receptor (ER) expression (73–94% versus 26–82%, supplementary material, Figure S5) and is associated with more progesterone receptor (PR) expression (63–90% versus 47–74%, supplementary material, Figure S6) [137–140,146–151]. For MC, less HER-2 overexpression has been reported compared with NMC (0–14% versus 20–41%, supplementary material, Figure S7) [139,140,146,147,149–151]. Studies that included small numbers of MC demonstrated that mutated *PIK3CA*, which is found in 16–33% of IDCs, is not a common finding in MC (0–13%), supplementary material, Figure S8 [125,152–157]. Mutations of *BRAF* and *KRAS* are not common in breast cancer (0–3% and 2–5%) and associations with MC have not been studied [65,158,159]. Unlike in colorectal MC, MSI is a rare phenomenon in MC of the breast, occurring only sporadically (0–3%) [160–165]. Studies evaluating *EGFR* mutations in breast cancer have not focused on MC.

Comparison with CRC

A common mucinous pathway cannot be identified for MC from different organs (Figure 5). However, in general limited data is available for non-colorectal MC. There are differences between MC and NMC in mutation rates of targets of the RAS/RAF/MAPK and PI3K/AKT pathways. Also differences in expression of EGFR, HER-2, ER and PR have been found in

non-colorectal MCs. The association between these molecular characteristics and the mucinous phenotype is not well studied in non-colorectal MC. However, *in vitro* studies with lung cancer cell lines showed that cell treatment with epidermal growth factor resulted in an increased expression of MUC2 [166]. Conversely, blockage of the PI3K/AKT pathway in gastric cancer cell lines resulted in an increase in MUC2 expression, indicating the need for further clarification of the regulatory mechanisms behind MUC2 expression in MCs [167].

MSI is another distinctive tumour characteristic of colorectal MC but has only been reported at a higher rate in MCs from the stomach and biliary tract. As various molecular characteristics have been associated with either worse or improved prognosis, differences in these pathways may explain deviant tumour behaviour of MC in different organs.

Conclusions and implications

The era of personalized medicine has led to an emerging interest in tumour subtypes and the molecular background of malignancies. The distinct clinicopathological presentation and the impaired response to systemic therapies are suggestive of a different molecular background of colorectal MC, but development of this subtype is not well understood. This review recapitulated alterations in several therapeutically important pathways of CRC and compared findings with the literature regarding MCs from other organs.

Overexpression of MUC2, leading to abundant mucin production, is a molecular key feature of MC, but it does not explain the distinct clinical behaviour of MC. Review of the literature demonstrated that MC showed higher rates of mutations in *BRAF*, *KRAS* and *PIK3CA* than NMC and higher rates of CIMP and MSI were found in MC. Funnel plots did not demonstrate publication bias (figures not shown). These findings suggest that mutations in the RAS/RAF/MAPK and PI3K/AKT pathways are involved in MC development.

Previously, it has been reported that MC is more commonly found in tumours arising under inflammatory conditions and in patients with a hereditary predisposition for CRC. A higher rate of MC was observed in patients suffering from inflammatory bowel diseases or Lynch syndrome and in patients who developed CRC following radiotherapy [19]. It is unknown to what extent these factors contribute to MC development, but they indicate that epigenetic changes may well influence MC development.

From a therapeutic perspective, colorectal MC has a worse outcome than NMC when treated with palliative chemotherapy for advanced stage disease [5–7]. Interestingly, there is no difference in benefit from adjuvant chemotherapy in MC patients [4,168]. MSI tumours have been associated with less responsiveness to 5-fluorouracil (5-FU) chemotherapeutic treatment [169], but this does not explain the discrepancy between the adjuvant and palliative setting. In rectal cancer, resistance of MC to radiotherapy or chemoradiotherapy is suspected, given the poorer rate of tumour downstaging [170,171]. Also, the metastatic pattern is different between MC and NMC patients [8]. This indicates that not only phenotype, but also tumour behaviour is different between histological subtypes.

As the definition of MC in CRC requires that at least 50% of the tumour consists of mucin, it is not inconceivable that tumour heterogeneity may have influenced findings from the literature. It is possible that molecular aberrations have remained unnoticed due to dilution by non-mucinous tumour elements. However, no study has attempted to address this problem by focusing solely on pure MC samples in CRC. Moreover, since CRC can develop via CIN and MSI it would be interesting to analyse molecular aberrations stratified by these different pathways. Unfortunately, this was not feasible as insufficient data were available in the literature.

This review also compared colorectal MC with MCs from other organs. The definition of MC is not unambiguous between different organs, as it sometimes refers to tumours containing abundant intracellular mucin or a combination of intracellular and extracellular mucin. MC is less prevalent in other organs than in the colorectum, which was reflected by the limited amount of literature on molecular differences between subtypes in these tumours. A common mucinous pathway could not be identified, but between MC and NMC, differences in mutation rates of components of the RAS/RAF/MAPK and PI3K/AKT pathways were found in most organs. Alterations in these pathways may be associated with MUC2 overexpression. Interestingly, the genetic instability pathway of MSI, which is a predominant characteristic of mucinous CRC, could not be linked to MCs in every other organ.

Further identification of molecular aberrations may lead to the development and implementation of targeted therapies but could also explain resistance of tumours to such therapies. Moreover, identification of the molecular background of MC may improve prognostication and could lead to a better prediction of response to local and systemic therapies.

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Author contributions

All authors were involved in writing the paper and had final approval of the submitted manuscript.

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SUPPLEMENTARY MATERIAL ON THE INTERNET

The following supplementary material may be found online.

Figure S1. Relative risk for CpG island methylator phenotype in studies comparing colorectal mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC).

Figure S2. Relative risk for microsatellite instability in studies comparing mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC) of the stomach.

Figure S3. Relative risk for *KRAS* mutation in studies comparing mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC) of the ovary.

Figure S4. Relative risk for microsatellite instability in studies comparing mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC) of the ovary.

Figure S5. Relative risk for oestrogen receptor expression in studies comparing infiltrating ductal carcinoma (ICD) and mucinous adenocarcinoma (MC) of the breast.

Figure S6. Relative risk for progesterone receptor expression in studies comparing infiltrating ductal carcinoma (ICD) and mucinous adenocarcinoma (MC) of the breast.

Figure S7. Relative risk for HER-2 expression in studies comparing infiltrating ductal carcinoma (ICD) and mucinous adenocarcinoma (MC) of the breast.

Figure S8. Relative risk for *PIK3CA* mutation in studies comparing infiltrating ductal carcinoma (ICD) and mucinous adenocarcinoma (MC) of the breast.

