Clinical and molecular aspects of MUTYH- and APC-associated polyposis

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MUTYH-associated polyposis carcinomas frequently lose HLA class I expression-a common event amongst DNA-repair-deficient colorectal cancers

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ABSTRACT

Human leukocyte antigen (HLA) class I expression defects frequently occur in colorectal cancers bearing mismatch repair (MMR) deficiencies and are interpreted as immune evasion mechanisms to avoid cancer cell recognition and elimination by the immune system. MMRdeficient tumours are thought to be more prone to lose HLA class I expression, due to their frequent generation of aberrant peptides which can stimulate a cytotoxic T-cell-mediated response. *MUTYH*-associated polyposis (MAP) is a colorectal cancer syndrome caused by defects in the MUTYH DNA repair enzyme. Impairment of *MUTYH* activity could lead to a surplus of mutated peptides which would be presented to cytotoxic T-cells through the HLA class I molecules. We have studied the frequency of HLA class I expression defects in MAP carcinomas and have compared it to those observed in MMR-deficient and -proficient colorectal tumours. Immunohistochemical detection of the expression of HLA class I, β2-microglobulin (β2m), and antigen-processing machinery molecules was performed in 37 primary MAP carcinomas and nine metastases resected from 29 MAP patients. Furthermore, we sequenced the β2m, TAP1, and TAP2 genes. Defects in HLA class I expression were detected in 65% of primary MAP carcinomas, affecting 72% of patients.

HLA class I expression abnormalities were often concomitant with β2m expression loss and mutations in the β2m gene. Loss of HLA class I expression is thus a frequent event in MAP carcinomas, similarly to MMR-deficient colorectal tumours. The extensive mutagenic background of these tumours most likely triggers a strong selective pressure, exerted by the immune system on the tumour, which favours the outgrowth of tumour cell clones with an immune evasive phenotype. Our data provide additional evidence for a link between DNA repair deficiencies and altered HLA class I phenotypes in colorectal cancer.

INTRODUCTION

During cancer development, tumour cells may elicit cytotoxic T-lymphocyte-mediated immune responses — partly a consequence of accumulated gene mutations that are translated into altered peptides.[1,2] Tumour cell expression of the human leukocyte antigen (HLA) class I-antigen complexes is essential for T-cell recognition of aberrant peptides and subsequent activation.[3] Consequently, alteration of HLA class I cell surface expression provides an effective mechanism by which tumours can escape detection by immune cells.[4,5] Multiple mechanisms have been shown to underlie defects in HLA class I expression by tumour cells; they include mutations in the individual HLA class I genes, *HLA-A,-B*, and *-C* , located on chromosome 6p21.3;[6] loss of heterozygosity (LOH) at 6p21.3;[7] mutations in β2-microglobulin (β2m),[8,9] the molecule required for the cell surface expression of HLA class I antigens; and defects in components of the HLA class I-associated antigen-processing machinery.[10–12] The antigen-processing machinery consists of the proteasome components delta, MB1, and Z; the immunoproteasome components LMP2, LMP7, and LMP10; the peptide transporters TAP1 and TAP2; and the chaperones calnexin, calreticulin, ERp57, and tapasin. The immunoproteasome mostly, although not exclusively, generates peptides from endogenous proteins. TAP1 and TAP2 facilitate peptide translocation from the cytosol into the lumen of the endoplasmic reticulum, where the peptides are loaded onto the HLA class I molecules with the aid of several chaperones.[13]

We and others have previously described a higher frequency of HLA class I defects in colorectal cancers presenting with DNA mismatch-repair (MMR) deficiency, in comparison with MMR-proficient tumours.[12,14,15] Furthermore, we found that distinct molecular events underlie HLA class I loss, depending on the aetiology of the tumours; Lynch syndrome-related cancers presented with mutations in the β2m molecule, while sporadic microsatelliteunstable tumours mainly showed alterations in the antigen-processing machinery components.[12]

Microsatellite instability is a form of genetic instability that occurs in about 15% of colorectal cancers. Microsatellite-unstable sporadic tumours have a phenotype resulting from the epigenetic inactivation of the MMR gene *hMLH1.*[16] Microsatellite instability is also the hallmark of Lynch syndrome-related tumours, in which germline mutations of the MMR genes *hMLH1, hMSH2, hMSH6,* and PMS2 can be found.[17] Their inactivation destroys a cell's ability to repair base–base mismatches and small insertions or deletions in repetitive stretches, leading to an accumulation of frameshift mutations that become translated into abnormal peptide sequences.[18] These tumours are therefore thought to be more able to stimulate a cytotoxic T-cell-mediated immune response, due to their frequent generation of aberrant peptides.[19,20] Consequently, these tumours are probably subjected to a selective pressure that favours the outgrowth of tumour cells with the ability to escape from recognition and destruction by the host immune system.

Another colorectal cancer syndrome in which DNA repair deficiencies are implicated is *MUTYH*-associated polyposis (MAP).[21] MAP is a recessive inheritable disease caused by bi-allelic *MUTYH* inactivating germline mutations.[22] Carriers may develop 10–500 polyps until the fifth decade of their lives and most of these patients will present with a colorectal cancer.[23,24] The MUTYH protein is a base excision repair enzyme involved in the repair of one of the most frequent and stable forms of nucleotide oxidative damage: 8-oxo-7,8-dihydro-2.deoxyguanosine (8-oxodG). This structure readily mispairs with adenosine residues, leading to G :C>T: A transversions after DNA replication.[25] MUTYH acts by scanning the daughter DNA strand for any mispaired adenines, either with guanines or 8-oxodG's, and subsequently excising them.[26] Defects in this repair mechanism incapacitate the cell's ability to reverse such mispairs, leading to an accumulation of mutations. The *APC* and *KRAS* genes are frequently mutated in MAP tumours, although not exclusively presenting the typical transversions.[21,27,28]

We hypothesized that, similarly to MMR-deficient tumours, MAP tumours could be more prone to stimulate a cytotoxic T-cell-mediated immune response, due to their frequent generation of aberrant peptides. Hence, these tumours could also be subjected to a strong selective pressure favouring the outgrowth of cancer cells that acquire an immune evasive phenotype.

We aimed to study the occurrence of HLA class I expression deficiencies in MAP carcinomas, as well as the expression of HLA class I chaperones and antigen-processing machinery components. Furthermore, we investigated the occurrence of mutations in β*2m* or antigen-processing machinery components when their expression was lost.

MATERIALS AND METHODS

Patient material

Patients were recruited throughout The Netherlands. Germline DNA mutation analysis of the whole *MUTYH* gene was performed on lymphocytic DNA or DNA from formalin-fixed, paraffin-embedded normal tissue as described previously.[29,30] Informed consent was obtained according to protocols approved by the LUMC ethics review board (02–2004). A tissue microarray was constructed as reported previously [31] and contained carcinoma tissue from 37 primary carcinomas and nine metastases (one lymph node and eight distant metastases), derived from 29 MAP patients, represented by more than four (mean = 4.5, SD = 1.7) 0.6-mm-diameter tissue cores. Such a number of tissue cores was shown to be sufficient to account for tumour heterogeneity and thus produce reliable results.[32] The tissue array also included 14 adenomas resected from the above patients. The cohort's age mean was 51.2 years old (SD = 9.9). The majority of primary carcinomas were localized in the right-sided colon (73%) and the remaining had a sigmoid/rectum localization. Tumours were staged according to Dukes' classification: one was Dukes' A, 22 B, six C, and six D (three patients; one patient presented with four primary tumours and one distant metastasis). Patient samples were handled according to the medical ethical guidelines described in the Code Proper Secondary Use of Medical Sciences (www.federa.org).

Immunohistochemistry

Standard three-step, indirect immunohistochemistry was performed on 4 μm tissue sections transferred to glass slides using a tape-transfer system (Instrumedics, Hackensack, NJ, USA), including citrate antigen retrieval (citrate buffer, pH 6.0), blockage of endogenous peroxidase and endogenous avidin-binding activity, and diaminobenzidine development.

The following primary antibodies were used: the monoclonal antibody (MAb) HCA2, which recognizes β2m-free HLA-A (except -A24), -B7301, and -G heavy chains;[33,34] the MAb HC10, which recognizes a determinant expressed on all β2mfree HLA-B and -C heavy chains and on β2mfree HLA-A10, -A28, -A29, -A30, -A31, -A32, and -A33 heavy chains [33,35]; TAP1-specific MAb NOB1; LMP2-specific MAb SY-1; LMP7-specific MAb HB2; LPM10-specific MAb TO-7; calnexinspecific MAb TO-5; calreticulin-specific MAb TO11; tapasin-specific MAb TO-3; ERp57-specific MAb TO-2;[36–38] TAP2-specific MAb (BD Biosciences Pharmingen, San Diego, CA, USA); rabbit anti-β2m polyclonal Ab (A 072; DAKO Cytomation, Glostrup, Denmark); and anti-granzyme B (clone 11F1; Novocastra Laboratories, Newcastle upon Tyne, UK). Secondary reagents used were biotinylated rabbit anti-mouse IgG antibodies (DAKO Cytomation), goat anti-rabbit IgG antibodies (DAKO Cytomation), and biotinylated-peroxidase streptavidin complex (SABC; DAKO Cytomation).

All antigen-processing machinery components' antibodies, except TAP2 MAb, were kindly provided by Dr Soldano Ferrone (Roswell Park Cancer Institute, Buffalo, NY, USA). Loss of expression was defined as a complete lack of staining in the membrane and cytoplasm (HCA2, HC10, and anti-β2m), in the peri-nucleus/endoplasmic reticulum (NOB1, anti-TAP2, TO-2, TO-3, TO-5, TO7, and TO-11), or in the cytoplasm (SY-1, HB2, and TO-7), but with concurrent staining in normal epithelium, stroma or infiltrating leukocytes.

β*2m*, *TAP1*,and *TAP2* sequencing

The β2m, *TAP1*, and *TAP2* genes were screened for mutations in their coding regions. DNA was isolated from three 0.6-mm-diameter tissue cores retrieved from a tissue block after evaluation of the corresponding haematoxylin and eosin-stained tissue slide. After dewaxing, the tissue cores were incubated overnight at 56 °C in 0.3 ng/μl proteinase K and purified the next day with the Genomic Wizard kit (Promega, Leiden, The Netherlands). PCR was performed in a25 μl volume containing 20 μM of primer pair and 12 ng of sample DNA. 18mer M13-derived oligos were added to the 5. end of each primer in order to facilitate sequence analysis. Primer sequences are available as Supplementary data (see Supporting information). Amplified products were sequenced at The Leiden Genome Technology Center (Leiden, The Netherlands).

RESULTS

HLA class I, β2m, and antigen-processing machinery components' expression

Defects in HLA class I expression were detected in 24/37 primary carcinomas and in all nine tumour metastases (seven of which derived from patient 29), comprising 72% of the patients included in the study (Table 1). The HCA2 and HC10 antibodies detect the expression of HLA-A and HLA-B, -C, respectively. Within the group of tumours with HLA class I defects, six primary carcinomas (25%) presented with total loss of HLA class I (concomitant loss of HCA2 and HC10); another two carcinomas (8%) presented with loss of solely the HLA-A molecule (as assessed with HCA2 antibody); and the remaining carcinomas (67%) presented heterogeneous patterns with alternating positive and negative tumour areas for HLA class I expression (Figure 1). All seven metastases from patient 29 expressed HLA class I, although no membranous expression was detectable with the HCA2 antibody (Figure 1).

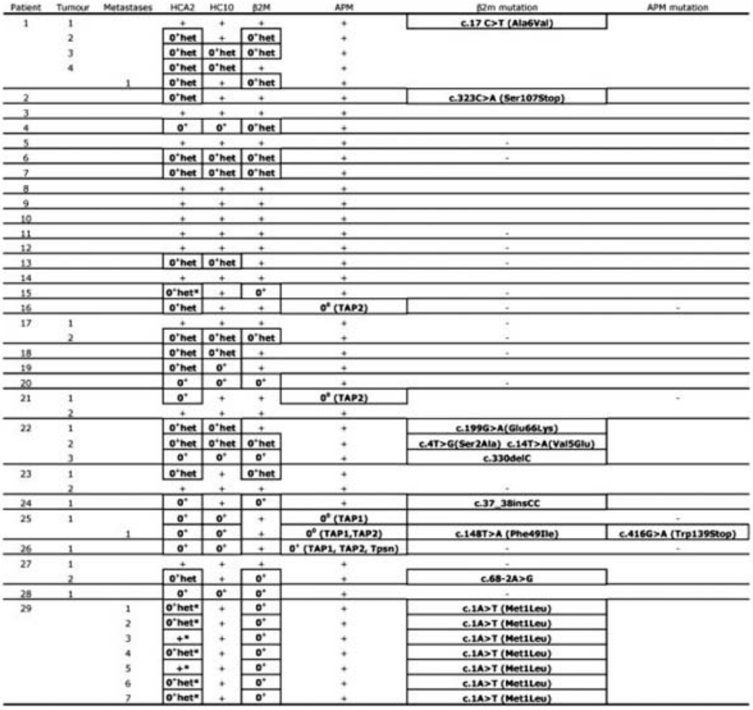
We also investigated the concurrent loss of expression of β2m and antigen-processing machinery components with that of HLA class I. Loss of β2m expression was detected in 58% of carcinomas with HLA class I expression abnormalities (Table 1); four of these presented with total loss of HLA class I (Figure 2B), two exclusively lost HLA-A expression, and eight cases possessed a heterogeneous pattern of HLA class I expression (Figures 2C and 2D) normally accompanied by a similar pattern of β2m expression. This heterogeneous pattern was frequently recognized within one tissue core, as represented in Figures 2C and 2D. Notably, the seven tumour metastases from patient 29, presenting with a peri-nuclear pattern of expression for HLA class I, showed total absence of β2m expression, further confirming the existence of HLA class I expression abnormalities (Figures 2E and 2F).

Table 1. Expression of HLA class I, β2m, and antigen-processing machinery components in MAP carcinomas

MAP carcinomas	HLA class I expression		β2m loss	APM loss
Primary tumours	+	13 (35.1%)	0	0
$n = 37$	−	24 (64.9%)	14 (58.3%)	4 (16.7%)
Metastases	+	0	0	0
$n = 9$*	−	9	8	1

− = altered expression in tumour cells.

* Seven of the nine metastases belonged to one patient.



Patient	Tumour	Metastases	HCA2	HC10	β2M	APM	β2m mutation	APM mutation
1	1		+	+	+	+	c.17 C>T (Ala6Val)	
	2		0^+het	+	0^+het	+		
	3		0^+het	0^+het	0^+het	+		
	4		0^+het	0^+het	+	+		
		1	0^+het	+	0^+het	+		
2			0^+het	+	+	+	c.323C>A (Ser107Stop)	
3			+	+	+	+		
4			0^+	0^+	0^+het	+		
5			+	+	+	+	-	
6			0^+het	0^+het	0^+het	+	-	
7			0^+het	0^+het	0^+het	+		
8			+	+	+	+		
9			+	+	+	+		
10			+	+	+	+		
11			+	+	+	+	-	
12			+	+	+	+	-	
13			0^+het	0^+het	+	+	-	
14			+	+	+	+		
15			0^+het*	+	0^+	+	-	
16			0^+het	+	+	0^0 (TAP2)	-	-
17	1		+	+	+	+	-	
	2		0^+het	0^+het	0^+het	+	-	
18			0^+het	0^+het	+	+	-	
19			0^+het	0^+	+	+		
20			0^+	0^+	0^+	+	-	
21	1		0^+	+	+	0^0 (TAP2)		-
	2		+	+	+	+		
22	1		0^+het	0^+het	+	+	c.199G>A(Glu66Lys)	
	2		0^+het	0^+het	0^+het	+	c.4T>G(Ser2Ala) c.14T>A(Val5Glu)	
	3		0^+	0^+	0^+	+	c.330delC	
23	1		0^+het	+	0^+het	+		
	2		+	+	+	+	-	
24	1		0^+	+	0^+	+	c.37_38insCC	
25	1		0^+	0^+	+	0^0 (TAP1)		-
		1	0^+	0^+	+	0^0 (TAP1,TAP2)	c.148T>A (Phe49Ile)	c.416G>A (Trp139Stop)
26	1		0^+	0^+	+	0^+ (TAP1, TAP2, Tpsn)	-	-
27	1		+	+	+	+	-	
	2		0^+het	+	0^+	+	c.68-2A>G	
28	1		0^+	0^+	0^+	+	-	
29		1	0^+het*	+	0^+	+	c.1A>T (Met1Leu)	
		2	0^+het*	+	0^+	+	c.1A>T (Met1Leu)	
		3	+*	+	0^+	+	c.1A>T (Met1Leu)	
		4	0^+het*	+	0^+	+	c.1A>T (Met1Leu)	
		5	+*	+	0^+	+	c.1A>T (Met1Leu)	
		6	0^+het*	+	0^+	+	c.1A>T (Met1Leu)	
		7	0^+het*	+	0^+	+	c.1A>T (Met1Leu)	

Figure 1. Expression of HLA class I was detected with HCA2 (HLA-A) and HC10 (HLA-B, HLA-C) antibodies. Additionally, the expression of the chaperone β2-microglobulin and the antigen-processing machinery components were determined. + = positive; 0^+ = loss of expression with internal positive control; 0^0 = loss of expression without internal positive control; 0^+ het = heterogeneous expression, with positive and negative tumour cells; $+^*$ = non-membranous (cytoplasmic HLA class I expression); 0^+ het* = heterogeneous expression, with positive (cytoplasmic HLA class I expression) and negative tumour cells. β2m mutations were investigated in all cases where DNA was available, and TAP1 and TAP2 mutations were screened when one of these molecules was not expressed. − =No mutations found with the complete gene sequence available

Concurrent loss of antigen-processing machinery components and HLA class I expression was observed in five cases: four primary tumours (17% of tumours with HLA class I expression abnormalities) and one tumour metastasis (Table 1). TAP1 and/or TAP2 were lost in all of these cases, while tapasin was found to be abrogated in patient 26 (Figure 1). Except for the latter, all other tumour tissues failed to present a clear internal positive control, thus not allowing the confirmation of loss of expression of those molecules. Loss of expression of the antigen-presenting machinery components and β2m was mutually exclusive.

Fourteen adenomas, from ten patients, were analysed for HLA class I and β2m expression. Only three (21%) displayed aberrant HLA class I expression which was always heterogeneous throughout the adenomatous tissue. All three belonged to patients with HLA class I deficiencies in at least one of their carcinomas. Loss of expression of β2m was found in one of the adenomas with HLA class I deficiencies.

β*2m*, *TAP1*,and *TAP2* sequencing

We sequenced the coding regions of the β2m, inall tumours for which DNA was available, and *TAP1* and *TAP2* genes in the tumours that lacked expression of these molecules. All mutations are listed in Figure 1. Mutations in β*2m* were identified in eight primary carcinomas. In one case (patient 1, tumour 1), the mutation was not related to loss of either β2m or HLA class I expression. Two other tumours conserved β2m expression but presented deficiencies in HLA class I expression (patient 2 and patient 22, tumour 1). The remaining all had detectable defective β2m expression. Eight metastases with HLA class I loss of expression carried a mutation in the β*2m* gene. All seven belonging to patient 29 presented with the same mutation in the starting codon of the β*2m* gene (c.1A > T). Starting-codon, splice-site or frameshift mutations were associated with loss ofβ2m expression. The carcinoma from patient 2 displayed a truncating mutation in the 3. end of the gene which did not lead to abrogation of β2m expression. Amino-acid substitutions were associated with retained β2m expression and in one case, a heterogeneous pattern of expression (patient 22, tumour 2). The complete β2m sequence was available for six additional tumours without HLA classI/β2m aberrations and no mutations were found. Only one mutation was found in the peptide transporters. Patient 25 presented an early STOP codon in the third exon of the *TAP2* gene. The usage of formalin-fixed material for DNA extraction did not always allow full gene sequencing for all cases, implying a possible underestimation of the number of samples carrying mutations.

DISCUSSION

Defects in HLA class I expression defects have been frequently reported in several cancers.[5,6,10,11,39] These are interpreted as mechanisms adopted by tumours to escape immune surveillance and thereby avoid tumour cell recognition and destruction. We, and others, have previously described a predisposition for MMR-deficient colorectal tumours to lose HLA class I expression.[12,14,15] MMR-deficient tumours are thought to be more competent at triggering an immune response, due to their increased mutagenic potential, when compared with MMR-proficient tumours, which translates into an accumulation of frameshift peptides that might work as tumour neo-antigens [8,19,20]. The immune reaction might function as a selective pressure that favours the outgrowth of tumour cell clones that have lost HLA class I expression. In the present work, we investigated the occurrence of HLA class I expression deficiencies in MAP carcinomas. Patients with MAP carry biallelic mutations in the *MUTYH* gene, which prevents the cells from accumulating point mutations derived from DNA oxidative damage. Like Lynch syndrome and sporadic MSI-H tumours, one would expect that MAP carcinomas would be competent at triggering immune responses and would be similarly subjected to a selective pressure, imposed by the immune system, favouring the outgrowth of cells with absent HLA class I expression.

We identified HLA class I expression abnormalities in the majority of MAP carcinomas analysed, both in the primary carcinomas and in the tumour metastases. In total, 72% of patients carried carcinomas (68% of primary tumours) that exhibited HLA class I expression deficiencies. Previously, we described that approximately half of MSI-H sporadic tumours and Lynch syndrome-related tumours had defects in HLA expression, while colon mismatch-repair-proficient tumours presented HLA class I expression abnormalities in only a minority of the carcinomas analysed (17%).[12] Put together, these observations provide additional evidence of the prevalence of HLA class I expression alterations in tumours carrying defects in DNA-repairing molecules. Furthermore, we analysed 14 adenomas from the same cohort of patients for HLA class I and β2m expression but these were seldom, as was previously reported for colorectal adenomas.[40] The increased mutagenic load and cytotoxic T-cell infiltration of carcinomas probably constitute an environment with greater selective pressure for immune evasive events than at adenoma stages.

Intriguingly, the majority of defects found in MAP carcinomas were in the form of heterogeneous patterns of HLA class I expression within the tumours, suggesting that MAP carcinomas are composed of different clones with respect to HLA class I expression. Such a phenotype was not observed in MSI-H tumours (data not shown).

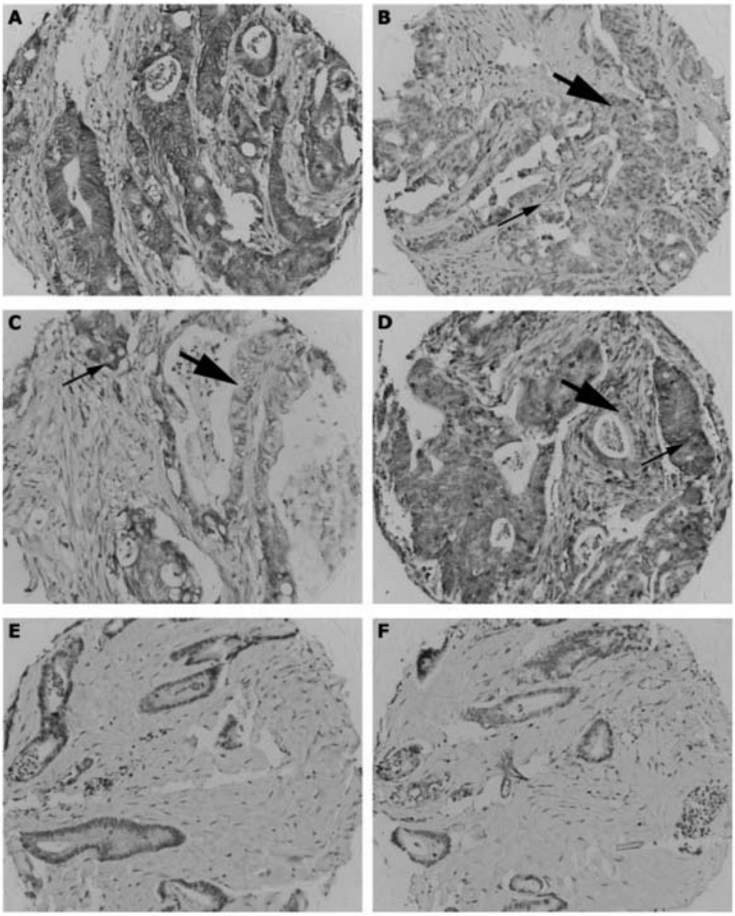


Figure 2. (A) Immunohistochemical detection of HLA class I expression with HCA2 antibody in the carcinoma from patient 11, displaying membranous expression in tumour cells. (B) HLA class I-negative carcinoma (large arrow) with positive internal control (small arrow) (patient 28). (C, D) Heterogeneous pattern of HLA class I expression; focal loss of HLA class I expression (large arrow) presented together with positive membranous expression (small arrow) (C, patient 17, tumour 2; D, patient 22, tumour 1). (E) Cytoplasmic accumulation of HLA class I in a case with β2m loss of expression, depicted in F (patient 29, metastases 4)

We previously reported that MAP carcinomas frequently display multiclonality for DNA content,[41,42] further supporting our observations. One piece of circumstantial evidence for increased immunogenicity of MMR-deficient tumours is the dense infiltration of the epithelial compartments of MSI-H carcinomas by T-cells, a hallmark of the histopathology of MMR-deficient tumours.[43,44] We also observed a higher incidence of moderate/marked infiltration by T-cells in the epithelium of MAP carcinomas, when compared with microsatellite-stable tumours (data not shown).

In the work of Dierssen *et al*, we dissected the most common mechanisms by which HLA class I expression was altered in sporadic MSI-H and hereditary (Lynch syndrome) MMR-deficient tumours.[12] We found that β*2m* mutations occurred preferentially in Lynch syndrome tumours, while sporadic MSI-H tumours presented more frequently with deficiencies at the level of the antigen-processing machinery components. Kloor *et al* also reported that mutations in the β2m gene occurred more frequently in Lynch syndrome-derived tumours than in sporadic MSI-H tumours.[9] We investigated whether the loss of any of these molecules was coupled to HLA class I expression deficiencies in MAP carcinomas; expression of β2m was absent in 58% of MAP primary carcinomas with altered HLA class I expression, while at least one of the antigen-processing machinery components was not detected in 18% of MAP carcinomas with HLA class I alterations. Although speculative, it is interesting to underline that carcinomas derived from both MAP and Lynch syndromes preferentially lose β2m expression coupled to HLA class I deficiencies. A functional explanation for these observations remains elusive, but perhaps distinct reactions (both qualitative and quantitative) by the immune system, depending on the age of onset of the tumours, could condition the type of mechanisms that lead to HLA class I expression deficiencies.

Middeldorp *et al* reported extensive chromosomal recombination events in MAP carcinomas.[41] These frequently translate into LOH with maintenance of chromosome copy number (copy neutral LOH) throughout the genome. LOH at chromosome 6p21 (*HLA locus*) and 15q22–23 (β*2m locus*) was frequently detected, but no association with HLA class Ior β2m expression was found (data not shown). In cases with maintenance of HLA class I expression, LOH at 6p might conceal an alternative HLA class I phenotype, not identified by immunohistochemistry: loss of one parental haplotype and amplification of the other. Each HLA class I haplotype has distinct affinities to different antigens, based on their peptide sequence. Therefore, the loss of one haplotype excuses the tumour cell from presenting a specific pool of peptides to the immune system. Moreover, the maintenance of one parental haplotype allows recognition of the 'self' by the immune cells and might impede the destruction of tumour cells by natural killer (NK) cells.[4] No polymorphisms in β2m have been associated with different efficiencies in peptide presentation; it is therefore difficult to determine whether LOH in 15q, in cases without HLA class I abnormalities, has any effect on HLA class I/antigen presentation.

The high frequency of alterations in HLA class I expression in MMR-deficient and in MAP carcinomas constitutes a strong handicap for the employment of Tcell-based immunotherapy on advanced tumours.[45] On the other hand, the fact that the immune system is able to recognize tumour antigens during cancer development reveals an opportunity for the development of vaccination strategies based on frequently altered pep-tides across different MAP carcinomas.[46,47] Additionally, memory T-cell responses could be used in the diagnostic setting, if they occurred at an early stage of tumour development, thus adding possibilities for the development of less invasive screening techniques, especially in carriers of hereditary syndromes.

We are the first to report HLA class I abnormalities in MAP carcinomas. It provides additional evidence that tumours carrying defects in DNA base repair mechanisms are more prone to undergo immune escape mechanisms. With this work, all known syndromes and subsets of sporadic colorectal tumours arising in the context of DNA repair deficiencies have now been linked with higher frequencies of HLA class I abnormalities, when compared with DNA base repair-proficient tumours.

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