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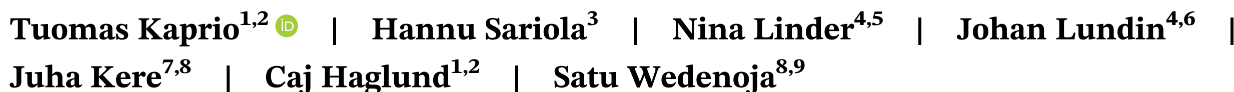
BRIEF COMMUNICATION

HLA Immune Response Genetics WILEY

HLA-G expression correlates with histological grade but not with prognosis in colorectal carcinoma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Trophoblast-specific expression of HLA-G induces immune tolerance for the developing fetus. Pathological HLA-G expression later in life might contribute to immune escape of various cancers. We studied the still controversial role of HLA-G in colorectal carcinoma (CRC) using the MEM-G/1 antibody and a tissue microarray series of CRC tumors ($n = 317$). HLA-G expression appeared in 20% of the tumors and showed high intratumoral heterogeneity. HLA-G positivity was associated with better differentiation ($p = 0.002$) and non-mucinous histology ($p = 0.008$). However, HLA-G expression alone showed no prognostic value: 5-years disease-specific survival among patients with HLA-G expression was 68.9% (95% CI: 62.7%–75.0%) compared to 74.8% (95% CI: 63.2%–86.3%) among those without expression. These results support a modulatory role of HLA-G in CRC.

HLA-G shows restricted physiological expression in immune privileged cells, such as invading extravillous trophoblasts of the human placenta. Through this restricted cell population, membrane-bound and soluble isoforms of HLA-G bind to their receptors, immunoglobulin-like transcript 2 (ILT2/LILRB1/CD85j), Immunoglobulin-like transcript 4 (ILT4/LILRB2/CD85d), and Killer Cell Immunoglobulin-Like Receptor 2DL4 (KIR2DL4) on maternal immune cells, and prevent the cells from attacking the semiallogenic fetus.[1,2] While physiological expression of HLA-G is mandatory for successful human pregnancy, HLA-G neoexpression later in life facilitates immune escape of both virus infected and cancer cells.[3,4] In colorectal carcinoma (CRC), HLA-G expression might correlate with tumor-node-metastasis (TNM) stage[5] but data on its effects on the overall survival remain

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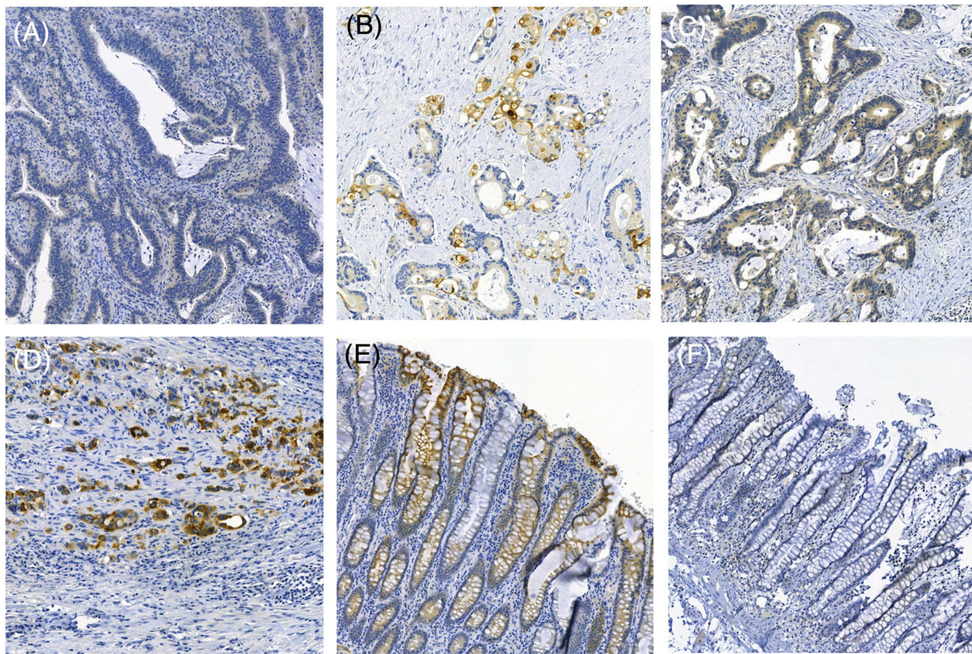

FIGURE 1 Immunohistochemical staining of HLA-G in colorectal cancer. Representative images of HLA-G expression in tissue microarrays of colorectal cancer. (A) negative HLA-G expression, (B) low HLA-G expression and also the mosaic-like expression pattern of HLA-G in colorectal cancer tissue, (C) high HLA-G expression, mosaic-like, (D) anaplasia of HLA-G positive tumor cells, which was often detected, (E) positivity and mosaic-like staining patterns seen in the tumor adjacent to the normal epithelium and (F) normal epithelium negative for HLA-G expression

conflicting.[2,6–14] We aimed to study HLA-G protein expression using a large tissue microarray series of CRC tumors ($n = 317$) and immunohistochemistry optimized for placental staining of HLA-G.[15] We further assessed HLA-G immunostaining in relation with clinical parameters associated with progressive disease and cluster of differentiation 3 (CD3) positive tumor infiltrating lymphocytes.

The study population of 317 CRC patients underwent surgery in 1998–2000 at the Helsinki University Hospital. At the time of the cohort collection, Dukes classification was used for cancer staging. The median age at diagnosis of CRC was 68 years, with a mean follow-up of 7.92 years (range 0–19). Follow-up vital-status data for survival analyses were provided by the Finnish Population Register Centre and causes of death by Statistics Finland. The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Helsinki University Hospital (Dnro HUS 226/E6/06, extension TMK02 §66 17.4.2013); the National Supervisory Authority of Welfare and Health gave the permission to use tissue samples without individual informed consent in this retrospective study (Valvira Dnro 10,041/06.01.03.01/2012).

Formalin-fixed and paraffin-embedded tumor samples were obtained from the pathology archives of the hospital. An experienced pathologist marked representative areas of tumor samples on hematoxylin- and eosin-stained tumor slides and two 1.0 mm diameter punches from each tumor block were mounted on recipient paraffin blocks with a semiautomatic tissue microarray instrument (Beecher Instruments, Silver Spring, MD, USA). The tumor tissue microarray (TMA) blocks were freshly cut into 4 μm sections, fixed on slides, and dried at 37°C

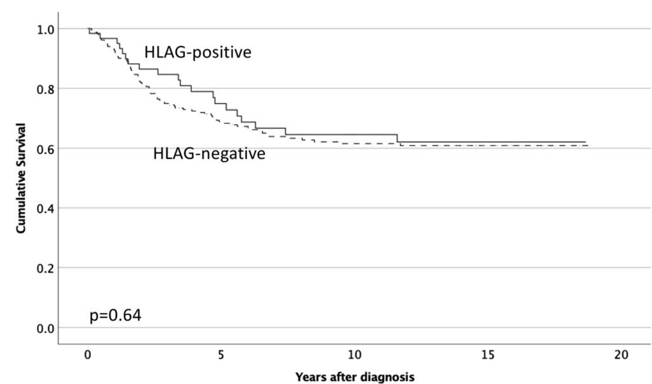

FIGURE 2 Disease-specific survival according to HLA-G expression in subjects with colorectal cancer

for 12 to 24 h. After deparaffinization in xylene and rehydration through a gradually decreasing concentration of ethanol to distilled water, TMA-slides were treated in a pretreatment module (Dako, Agilent, Santa Clara, CA, USA) in antibody-specific buffer for 15 min at 98°C for antigen retrieval. The sections were stained in an Autostainer 480 (Lab Vision AB, Värmdö, Sweden) by the Dako REAL EnVision Detection system, Peroxidase/DAB+, Rabbit/Mouse (Dako). Tissues were incubated with the HLA-G primary antibody (MA1-19219; Thermo Fischer Scientific, Waltham, MA, USA) at a dilution of 1:100 overnight at room temperature. For CD3, the sections were stained on the Ventana BenchMark Ultra instrument (Roche, Basel, Switzerland) and Ventana Ultraview DAB detection system (Roche). Tissues were incubated with the ready-to-use CONFIRM CD3 antibody (2GV6; Roche) for 40 min at room temperature.

The cytoplasmic expression of HLA-G in tumor cells was scored as either negative, low or high according to the intensity. The highest score of each sample was considered representative for the expression of HLA-G. For statistical analysis, samples were grouped into negative and positive. Stainings were scored independently by

TABLE 1 Association between HLA-G expression and clinicopathological parameters

HLA-G			
	Negative	**Positive**	***p* value**[a]
N (%)	246 (79.6)	63 (20.4)	
Age (median, IQR)	68.7 (58.2–76.7)	66.1 (57.5–76.7)	0.42
Gender			
Male	129 (52.4)	32 (50.8)	0.82
Female	117(47.6)	31 (49.2)	
Dukes staging			
A	44 (17.9)	13 (20.6)	0.52
B	71 (28.9)	19 (30.2)	
C	86 (35.0)	21 (33.3)	
D	45 (18.3)	10 (15.9)	
T class			
1	9 (3.7)	2 (3.2)	0.76
2	56 (22.8)	15 (23.8)	
3	155 (63.0)	39 (61.9)	
4	26 (10.6)	7 (11.1)	
N class			
0	125 (50.8)	35 (55.6)	0.66
1	72 (29.3)	15 (23.8)	
2	49 (19.9)	13 (20.6)	
Grade (WHO)			
1–2	202 (82.4)	62 (98.4)	**0.002**
3–4	43 (17.6)	1 (1.6)	
Missing	1		
Location			
Colon	116 (47.2)	30 (47.6)	0.95
Rectum	130 (52.8)	33 (52.4)	
Side			
Right	71 (28.9)	15 (23.8)	0.43
Left	175 (71.1)	48 (76.2)	
Histology			
Non-mucinous	221 (89.8)	63 (100)	**0.008**
Mucinous	25 (11.2)	0 (0)	
Preoperative CRP			
≤30	206 (84.4)	53 (86.9)	0.63
>30	38 (15.6)	8 (13.1)	
Missing	2	2	

Note: *P* values <0.05 are shown bolded.

Abbreviations: CRP, C-reactive protein; IQR, interquartile range. T class, the extent (size) of the tumor. N class, the spread to nearby lymph nodes. WHO, World Health Organization.

[a]Chi-square test or linear-by-linear association test for categorical variables, and Mann–Whitney *U* test for continuous variables.

Tuomas Kaprio and Hannu Sariola, who were blinded to clinical data and outcome. Differences in scoring were discussed until consensus. Evaluation of the association between HLA-G expression and clinicopathological parameters was performed by the Pearson exact Chi-square test or the exact linear-by-linear association test for categorical variables, and Mann-Whitney *U* test for continuous variables. Disease-specific survival (DSS) was counted from the date of surgery to the date of death from CRC, or until end of follow-up. The log rank test compared survival by the Kaplan–Meier method. All tests were two-sided. P-values of <0.05 were considered significant. All statistical analyses were done with SPSS version 27.0 (IBM SPSS Statistics, version 27.0 for Mac; SPSS, Inc., Chicago, IL).

Of the 317 tumors represented in the TMA, HLA-G staining could be evaluated in 309 (97.5%): 246 (79.6%) were scored as negative, 43 (13.9%) as low expression, and only 20 (6.5%) as high expression. HLA-G expression in tumor cells was mainly cytoplasmic. Interestingly, staining was often patchy, with distinctive negative and positive areas next to each other within the same tumor (Figure 1).

HLA-G positivity was associated with better differentiation ($p = 0.002$) and non-mucinous histology ($p = 0.008$). In the entire series, the 5-year disease-specific survival (DSS) rate was 68.9% (95%Cl 63.3%–73.3%). We observed no statistical difference in the prognosis according to HLA-G expression: the 5-year DSS among patients with positive HLA-G tumor expression was 68.9% (95% CI 62.7%–75.0%) compared to 74.8% (95% CI 63.2%–86.3%) among those with negative expression (Figure 2). No association emerged with age, gender, stage, systemic inflammation (preoperative C reactive peptide values), or localization (colon vs. rectum or right vs. left) (Table 1).

When whole tissue sections of 10 tumors with positive HLA-G staining in the TMA-series were stained with both HLA-G and CD3 antibodies, we confirmed a focal expression pattern within the same tumor. HLA-G positive cells tended be larger and anaplastic by histological criteria. There was no clear trend between HLA-G positive cells and surrounding CD3-positive immune cells nor with HLA-G-positivity and the invasive front. Normal epithelium next to malignant glands was HLA-G negative, with one exception where positivity was also patchy/focal (Figure 1).

Our findings are in line with previous studies, with many of them failing to show prognostic value of HLA-G expression in CRC.[4,16] However, our data indicated that non-mucinous histology, but not mucinous,[17] might be associated with HLA-G expression. In contrast, we found no evidence of HLA-G expression in association with poor differentiation,[5,18,19] but more tumors with the lowest grades of 1 to 2 were positive for HLA-G expression. This finding might be related to the low number of samples with high grade in this study, or with the overall spatial staining patterns and low number of samples positive for HLA-G. Remarkably, the observed pattern of HLA-G expression is in line with high intratumor heterogeneity of HLA-G expression in various cancers, including CRC.[4,12,16,20] However, we found no evidence that the spatial localization of HLA-G expression would be associated with surrounding CD3-positive immune cells or the invasive border of the tumor.

We found that only 20% of the tumors were positive for HLA-G expression. This finding is in line with the previous study on CRC showing 5%–28% of cells positive with the MEM/G-1 antibody.[16] The MEM-G1 antibody recognizes the denatured form of the HLA-G heavy chain, all isoforms,[14,21] and the native HLA-G2.[22] The observed pattern of HLA-G expression in this study supported the overall low expression of HLA-G in up to 70% of CRC tumors and lack of association between the positive staining and prognosis.[12] However, high discrepancies between the studies and rate of positive immunostaining with different antibodies of up to 65% exist.[1,7,8,13] Moreover, soluble HLA-G, which might be impossible to detect from tissue sections, may associate with CRC disease stage.[17]

Our study, which includes a significant number of CRC subjects and their follow-up data, fails to show any association between HLA-G staining and the survival in CRC. While it remains possible that only strongly positive immunoreactivity for HLA-G shows association with prognosis,[12] temporal and spatial differences in expression, possibilities of non-specific binding of HLA-G by different antibodies[23–25] and the lacking murine homolog of HLA-G provide challenges for studies on this immune escape molecule in the context of cancer, specifically CRC. Whether differentiation or invasive capacity of the cancer cells, known to upregulate HLA-G expression,[2,26] or clinically relevant features promote mosaic-like/focal staining patterns remain to be clarified.

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CONFLICTS OF INTEREST

The authors confirm that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Nina Linder, Johan Lundin, Juha Kere, Caj Haglund and Satu Wedenoja designed the study. Caj Haglund collected the patient cohorts and prepared the samples. Tuomas Kaprio, Hannu Sariola and Satu Wedenoja generated and analyzed the data. All authors interpreted the data. Tuomas Kaprio and Satu Wedenoja wrote the manuscript and all authors substantially revised the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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