Active immunosurveillance in the tumor microenvironment of colorectal cancer is associated with low frequency tumor budding and improved outcome

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Tumor budding (single tumor cells or small tumor cell clusters) at the invasion front of colorectal cancer (CRC) is an adverse prognostic indicator linked to epithelial-mesenchymal transition. This study characterized the immunogenicity of tumor buds by analyzing the expression of the major histocompatibility complex (MHC) class I in the invasive tumor cell compartment. We hypothesized that maintenance of a functional MHC-I antigen presentation pathway, activation of CD8+ T-cells, and release of antitumoral effector molecules such as cytotoxic granule-associated RNA binding protein (TIA1) in the tumor microenvironment can counter tumor budding and favor prolonged patient outcome. Therefore, a well-characterized multipunch tissue microarray of 220 CRCs was profiled for MHC-I, CD8, and TIA1 by immunohistochemistry. Topographic expression analysis of MHC-I was performed using whole tissue sections (n = 100). Kirsten rat sarcoma viral oncogene homolog (KRAS) and B-Raf proto-oncogene, serine/threonine kinase (BRAF) mutations, mismatch repair (MMR) protein expression, and CpG-island methylator phenotype (CIMP) were investigated. Our results demonstrated that membranous MHC-I expression is frequently down-regulated in the process of invasion. Maintained MHC-I at the invasion front strongly predicted low-grade tumor budding ($P = 0.0004$). Triple-positive MHC-I/CD8/TIA1 in the tumor microenvironment predicted early T-stage ($P = 0.0031$), absence of lymph node metastasis ($P = 0.0348$), lymphatic ($P = 0.0119$) and venous invasion ($P = 0.006$), and highly favorable 5-year survival (90.9% vs 39.3% in triple-negative patients; $P = 0.0032$). MHC-I loss was frequent in KRAS-mutated, CD8+ CRC ($P = 0.0228$). No relationship was observed with CIMP, MMR, or BRAF mutation. In conclusion, tumor buds may evade immune recognition through downregulation of membranous MHC-I. A combined profile of MHC-I/CD8/TIA1 improves the prognostic value of antitumoral effector cells and should be preferred to a single marker approach. (Translational Research 2015;166:207–217)

Abbreviations: BRA = B-Raf proto-oncogene, serine/threonine kinase; CD = Cluster of Differentiation; CDKN2A = Cyclin-dependent kinase inhibitor 2A; CI = Confidence interval; CIMP = CpG island methylator phenotype; CRC = Colorectal cancer; HLA = Human leucocyte antigen;

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HPF = High-power field; HR = Hazard ratio; KRAS = Kirsten rat sarcoma viral oncogene homolog; MHC = Major histocompatibility complex; MLH1 = mutL homolog 1; MMR = Mismatch repair; MSH2 = mutS homolog 2; MSH6 = mutS homolog 6; MSI = Microsatellite instability; NEUROG1 = Neurogenin 1; NK-cell = Natural Killer cell; PMS2 = PMS2 postmeiotic segregation increased 2; REMARK = REporting recommendations for tumour MARKer prognostic studies; ROC = Receiver Operating Characteristic; RUNX3 = Runt-related transcription factor 3; SOCS1 = Suppressor of cytokine signalling 1; TAP = Transporter associated with antigen processing; TAP1 = Transporter 1; ATP-binding cassette = sub-family B; TAP2 = Transporter 2; ATP-binding cassette = sub-family B; TIA1 = Cytotoxic granule-associated RNA binding protein; TMA = Tissue microarray

AT A GLANCE COMMENTARY

Koelzer VH, et al.

Background

Tumor-host interaction is an important field of research regarding prognosis in colorectal cancer (CRC). Single cell invasion (tumor budding) is an independent adverse prognostic indicator linked to epithelial-mesenchymal transition of CRC in experimental studies. This study characterized the immunogenicity of tumor buds in a well-characterized patient cohort by analyzing the expression of the major histocompatibility complex (MHC) class I, cytotoxic T-cell infiltration, and activation in the invasive tumor cell compartment.

Translational Significance

We illustrate that analysis of a comprehensive profile of MHC-I/CD8/TIA1 in the tumor microenvironment improves the prognostic value of antitumoral effector cells and should be preferred to a single marker approach.

INTRODUCTION

Tumor-host interaction is an important field of investigation regarding prognosis in colorectal cancer (CRC). Evidence is accumulating that the adaption of the immune response to the dynamic mutational landscape of CRC centrally contributes to the clinical behavior of disease.[1,2] A 3-step process is essential to mount an adaptive antitumoral immune response by the host: this includes the recognition of tumor-associated antigens bound to major histocompatibility complex class I (MHC-I), the activation of CD8+ T-cells, and destruction of tumor cells by cytotoxic effector molecules such as cytotoxic granule-associated RNA binding protein (TIA1).[2,3] The presence of peri- and intratumoral CD8+ T-cells has previously been identified as a major prognostic factor in patients with CRC.[4,5] However, tumor cells can escape immune recognition through loss or alteration of MHC-I molecules on the cell surface leading to reduced immunogenicity and the generation of immune escape variants in CRC.[6]

A common mechanism for MHC loss is genetic alteration in the antigen presentation pathway such as inactivation of β2-microglobulin and downregulation of transporters associated with antigen presentation (TAP).[7] This has frequently been observed in CRCs following the chromosomal and microsatellite instability pathway.[8-10] Less is known so far about the impact of aberrant methylation on MHC expression, particularly in the context of the CpG island methylator phenotype (CIMP) of CRC.[11]

In carcinomas of the intestinal tract, epithelial-mesenchymal transition is a signature of aggressive disease.[12] Histomorphologically, this phenotype can be observed as the presence of tumor budding, a feature strongly associated with lymphatic invasion and nodal metastasis as well as venous invasion and distant metastasis.[13] It is thought that tumor buds may possess stem-cell–like features and resemble cancer-initiating cells.[12] In the context of molecular pathology features, tumor budding is most frequently seen in B-Raf proto-oncogene, serine/threonine kinase (BRAF)-mutated CRC and is commonly absent in microsatellite instable CRC.[14] Tumor budding has been shown to be inversely correlated to CD8+ T-cell infiltration, suggesting a possible T-cell based defense against infiltrating tumor-budding cells in the tumor microenvironment of CRC.[15] However, little is known on how tumor buds evade the immune response during invasion and if oncogenic driver mutations may contribute to this process.

To address this question, we performed a novel topographic analysis of MHC-I expression in CRC including tumor center, tumor front, and tumor buds. Furthermore, we aimed to analyze the impact of molecular features