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Clinical impact of programmed cell death ligand 1 expression in colorectal cancer

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Abstract ***Background:*** Programmed cell death 1 (PD-1) receptor triggering by PD ligand 1 (PD-L1) inhibits T cell activation. PD-L1 expression was detected in different malignancies and associated with poor prognosis. Therapeutic antibodies inhibiting PD-1/PD-L1 interaction have been developed.

Materials and methods: A tissue microarray ($n = 1491$) including healthy colon mucosa and clinically annotated colorectal cancer (CRC) specimens was stained with two PD-L1 specific antibody preparations. Surgically excised CRC specimens were enzymatically digested and analysed for cluster of differentiation 8 (CD8) and PD-1 expression.

Results: Strong PD-L1 expression was observed in 37% of mismatch repair (MMR)-proficient and in 29% of MMR-deficient CRC. In MMR-proficient CRC strong PD-L1 expression correlated with infiltration by $CD8^+$ lymphocytes ($P = 0.0001$) which did not express PD-1. In univariate analysis, strong PD-L1 expression in MMR-proficient CRC was significantly associated with early T stage, absence of lymph node metastases, lower tumour grade, absence of

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vascular invasion and significantly improved survival in training ($P = 0.0001$) and validation ($P = 0.03$) sets. A similar trend ($P = 0.052$) was also detectable in multivariate analysis including age, sex, T stage, N stage, tumour grade, vascular invasion, invasive margin and MMR status. Interestingly, programmed death receptor ligand 1 (PDL-1) and interferon (IFN)-γ gene expression, as detected by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) in fresh frozen CRC specimens ($n = 42$) were found to be significantly associated ($r = 0.33$, $P = 0.03$).

Conclusion: PD-L1 expression is paradoxically associated with improved survival in MMR-proficient CRC.

1. Introduction

Tumour-infiltrating lymphocytes (TILs) are widely considered to reflect primary host immune response against solid tumours. Recent reports have demonstrated a direct correlation between colorectal cancer (CRC) patient survival and tumour infiltration by cluster of differentiation 8 (CD8) positive T lymphocytes expressing typical activation markers.[1,2] However, the immune system is characterised by the presence of a number of inhibitory mechanisms preventing 'excessive' lymphocyte activation.[3] In particular, programmed cell death receptor 1 (PD-1; CD279) is typically expressed by activated lymphocytes.[4] Its engagement by specific ligands, including PD ligand 1 (PD-L1; B7-H1; CD274) and PD ligand 2 (PD-L2; B7-DC; CD273), induces down-regulation of antigen-stimulated lymphocyte proliferation[5,6] and cytokine production,[6,7] ultimately resulting in lymphocyte 'exhaustion' and in the induction of immunological tolerance.[6,8–10]

PD-L1 is constitutively expressed by T and B cells, macrophages and dendritic cells (DC) and is up-regulated upon activation by interferons (IFN).[8,9] PD-L1 is also expressed on additional cell types including endothelial, pancreatic and muscle cells.[4] In contrast, PD-L2 expression is much more restricted and typically detectable in activated DC and macrophages.[9] Importantly, up-regulation of the expression of PD-1 ligands in malignant cells has been suggested to play a central role in tumour-immune system interaction[5,11] since, by triggering PD-1, cancer cells might shut down specific immune responses. Indeed, the expression of PD ligands on tumour cells was shown to suppress the cytolytic activity of $CD8^+$ T-cells.[12,13]

PD-L1 and, to a lesser extent, PD-L2, have been reported to be expressed by tumour cells of different origins, including glioblastoma, ovarian and renal cell carcinomas, squamous cell carcinoma of the head and neck, oesophageal and non-small cell lung cancers.[5,14–18] A strong correlation between expression of PD ligands on tumour cells and severe prognosis has been observed in oesophageal cancer and in renal cell carcinoma.[15,17] Capitalising on this background, PD-1/PD-L1 blockade by anti PD-1 or anti PD-L1 monoclonal antibodies has been envisaged as an appealing option to activate the host immune system to eradicate tumours. Recently, promising results of phase I clinical trials involving patients bearing a variety of malignancies have been published.[19–21]

Expression of PD-L1 in human CRC has not been addressed so far. In this study we used a tissue microarray (TMA)[22] including 1420 well documented, clinically annotated CRC specimens[23] to investigate the expression of PD-L1 in CRC and its clinical significance.

2. Materials and methods

2.1. Tissue microarray construction

The TMA used for this study includes 1420 unselected, non-consecutive, primary, sporadic CRCs treated between 1987 and 1996, and 71 normal mucosa specimens from the Institute of Pathology of the University of Basel (Switzerland), the Institute of Clinical Pathology, Basel (Switzerland) and the Institute of Pathology of the Stadtspital Triemli, Zürich (Switzerland). TMA was constructed with materials collected from the Tissue Biobank of the Institute of Pathology, University Hospital Basel. This institution performs translational research with the approval of the EKBB (Ethics Committee Beider Basel) in compliance with ethical standards and patient confidentiality. Construction of this TMA has been previously described in detail.[23] Briefly, formalin-fixed, paraffin-embedded tissue blocks from resected CRC were obtained. Tissue cylinders with a 0.6 mm diameter were punched from representative tissue areas of each donor tissue block and brought into one recipient paraffin block (30 × 25 mm). Each TMA spot included at least 50% tumour cells.

2.2. Immunohistochemistry

Four micron sections of TMA blocks were transferred to an adhesive-coated slide system (Instrumedics Inc., Hackensack, NJ, United States of America (USA)). Standard indirect immunoperoxidase procedures were used for immunohistochemistry (IHC; ABC-Elite, Vector Laboratories, Burlingame, CA, USA). Briefly, slides were dewaxed and rehydrated in distilled water. Endogenous peroxidase activity was