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Research paper

Surface antigen profiling of colorectal cancer using antibody microarrays with fluorescence multiplexing

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ABSTRACT

A procedure is described for the disaggregation of colorectal cancers (CRC) and normal intestinal mucosal tissues to produce suspensions of viable single cells, which are then captured on customized antibody microarrays recognising 122 different surface antigens (DotScan™ CRC microarray). Cell binding patterns recorded by optical scanning of microarrays provide a surface profile of antigens on the cells. Sub-populations of cells bound on the microarray can be profiled by fluorescence multiplexing using monoclonal antibodies tagged with Quantum Dots or other fluorescent dyes. Surface profiles are presented for 6 CRC cell lines (T84, LIM1215, SW480, HT29, CaCo and SW620) and surgical samples from 40 CRC patients. Statistical analysis revealed significant differences between profiles for CRC samples and mucosal controls. Hierarchical clustering of CRC data identified several disease clusters that showed some correlation with clinico-pathological stage as determined by conventional histopathological analysis. Fluorescence multiplexing using Phycoerythrin- or Alexa Fluor 647-conjugated antibodies was more effective than multiplexing with antibodies labelled with Quantum Dots. This relatively simple method yields a large amount of information for each patient sample and, with further application, should provide disease signatures and enable the identification of patients with good or poor prognosis.

1. Introduction

After resection of the primary colorectal cancer (CRC), 30–50% of patients relapse and succumb to systemic disease within 5 years (Weitz et al., 2005). Current prognosis and classification of CRC depends on staging systems that integrate histopathologic and clinical findings. In an attempt to prevent recurrences, adjuvant chemotherapy is administered routinely to patients classified with stage III or high-risk stage II CRC by Australian Clinico-Pathological (ACP) staging criteria; however as few as 10–20% of patients will benefit (Prall et al., 2004). This is because the clinical course

Abbreviations: CRC, Colorectal cancer; CD, Cluster of differentiation; TILs, Tumour-infiltrating lymphocytes; TAMs, Tumour-associated macrophages; IHC, Immunohistochemistry; QDs, Quantum Dots; FCS, Foetal calf serum; ACP, Australian Clinico-Pathological Staging; BSA, Bovine serum albumin; HBSS, Hanks' balanced salt solution; MeV, MultiExperiment Viewer; CEA, Carcinoembryonic antigen; EpCAM, Epithelial cell adhesion molecule; EGFR, Epidermal growth factor receptor.

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for individuals remains difficult to predict within the same-stage tumour categories. In the majority of CRC cases, cell dysfunction is the result of numerous mutations that modify protein expression and post-translational modification (Steinert et al., 2002). A number of cell surface antigens, including cluster of differentiation (CD) antigens, have been identified as potential prognostic or metastatic biomarkers in CRC (Table 1). These antigens make ideal biomarkers as their expression often changes with tumour progression or interactions with other cell types, such as tumour-infiltrating lymphocytes (TILs) and tumour-associated macrophages (TAMs). Although the accumulation of activated TILs and TAMs within the tumour has been linked to improved patient survival, the prognostic significance of these immune/inflammatory infiltrates in CRC remains controversial (Lewis and Pollard, 2006; Laghi et al., 2009).

The use of immunohistochemistry (IHC) for cancer sub-classification and prognostication is well established for some tumour types (Eifel et al., 2001; Swerdlow et al., 2008). Currently for CRC, the use of IHC is generally confined to the assessment of the expression of DNA microsatellite mismatch repair (MMR) protein in selected patients to identify a subgroup of tumours (10–15% of cases) that may be associated with a familial cancer syndrome (hereditary non-polyposis colorectal cancer),

Table 1
Panel of selected CD antigens and other cell surface proteins that may serve as biomarkers of disease progression and metastasis in CRC.

Antigen	Importance in CRC
CD9	Metastasis suppressor. Decreased or negative expression correlates with higher frequency of venous vessel invasion and metastasis (Le Naour et al., 2006)
CD10	Expression in CRC is a good predictor of liver metastasis (Fujimoto et al., 2005)
CD13	Expression correlates with reduced disease-free and overall survival (Hashida et al., 2002)
CD15s	Expression correlates with increased risk for metachronous distant spread (Yamada et al., 1995)
CD24	Expression correlates with shortened patient survival and nodal or systemic metastasis (Weichert et al., 2005)
CD29	Appears to be involved in increase of metastatic activity (Okazaki et al., 1998)
CD44	Important in tumour invasiveness, cell migration, angiogenesis. May act as tumour promoter or suppressor (Ngan et al., 2007)
CD44-v6	Presence of CD44-v6 in primary tumour is associated with poor prognosis (Wielenga et al., 1993)
CD47	Upregulated in some cancers; may participate in promotion of growth and metastasis (Shinohara et al., 2006)
CD49f	Strong correlation with CRC differentiation, invasive properties and metastatic abilities (Le Naour et al., 2006)
CD55	May be involved in tumour escape mechanisms by protecting tumours against lysis by activated complement (Durrant et al., 2003)
CD59	May be involved in tumour escape mechanisms by protecting tumours against lysis by activated complement (Watson et al., 2006a)
CD63	Appears to have a suppressor role, limiting tumour invasion and progression (Sordat et al., 2002)
CD66e	Participates in progression and metastatic growth of CRC. Not prognostic in primary tumour, but may be predictive in lymph node metastases (Ishida et al., 2004)
CD69	Early activation marker on infiltrating T-cells, NKT cells and macrophages in CRCs (Koch et al., 2006)
CD82	Metastasis suppressor. Decreased expression in CRC correlates with higher frequency of venous vessel invasion and metastasis (Le Naour et al., 2006)
CD87	Increased expression correlates with tumour progression, distant metastases, tumour recurrence and shortened disease-free or overall survival (Ge and Elghetany, 2003)
CD95	May be an independent prognostic factor in colon carcinoma. Diminished or abrogated in 40–50% of carcinomas (Strater et al., 2005)
CD98	Expressed on proliferating T-cells and many malignant or transformed cells; can suppress T-cell proliferation (Diaz et al., 1997)
CD104	Part of the tetraspanin web which has been linked to colon cancer metastasis (Le Naour et al., 2006)
CD151	Overexpression in CRC increases metastatic potential; correlated with poor prognosis (Le Naour et al., 2006)
CD166	Independent prognostic marker; membrane expression correlates with significantly shorter survival time (Weichert et al., 2004)
CD175s	Associated with poor clinical outcome (Itzkowitz et al., 1989)
CD227	Important predictor of metastatic potential and prognosis of colorectal cancer (Hiraga et al., 1998)
CD244	MHC Class I molecule; differentially expressed in CRC and normal mucosa (Jankova et al., 2009)
CD26	Sometimes aberrantly expressed in colon tumours; important role in immune regulation and tumour progression (Le Naour et al., 2006)
CD261	Extensive expression in CRC; downregulation of CD261 (Death Receptor 4) is associated with poor prognosis (Strater et al., 2002)
CD262	Stronger expression of Death Receptor 5 in CRC than normal mucosa correlates with higher apoptosis (Koornstra et al., 2003)
CD324	Potent tumour suppressor. Low expression of CD324 (E-cadherin) associated with a shorter survival rate (Ngan et al., 2007)
CD326	Overexpressed by majority of epithelial carcinomas. Prognostic significance controversial, but target for therapy (Chaudry et al., 2007)
CD340	Possible correlation with differentiation, Dukes classification and relapse-free and post-operative survival (Park et al., 2004)
β-Catenin	Altered distribution linked to poorer survival; may serve as a potential marker for progression and prognosis (Chen et al., 2008)
Annexin II	May be related to progression and spread of CRC; may have prognostic significance (Singh, 2007)
CA 125	Elevated in CRC; might contribute to metastasis (Mavligit and Estrov, 2000)
Claudin-4	Upregulated in CRC; decrease at invasive front associated with cancer invasion and metastasis (Ueda et al., 2007)
DCC	Tumour suppressor. Independent prognostic factor; absence of DCC linked to poor survival in Dukes stage B2 (Shibata et al., 1996)
EGF-R	Correlation with Dukes stage, differentiation and survival (Francoual et al., 2006)
FAP	High levels associated with higher likelihood of aggressive disease progression and development of metastases (Henry et al., 2007)
Galectin-3	Expression may be an independent factor for prognosis in colorectal cancer (Endo et al., 2005)
Galectin-4	Significant prognostic value in Dukes A & B. Increase in expression favours cell migration and metastasis (Nagy et al., 2003)
Galectin-8	Tumour suppressor activity. Prognostic in Dukes C & D (Nagy et al., 2003)
HLA-A,B,C	Down-regulation of HLA class I in rectal cancer is associated with poor prognosis (Speetjens et al., 2008)
HLA-DR	Strong HLA-DR antigen expression on cancer cells relates to better prognosis of colorectal cancer patients (Matsushita et al., 2006)
MICA	Partial loss confers poor prognosis in CRC (Watson et al., 2006b)
MMP14	Overexpression associated with tumour invasiveness in CRC (Malhotra et al., 2002)
PIGR	Highly expressed in normal colon epithelium but downregulated in CRC (Traicoff et al., 2003)
CA 19-9	Useful marker (Sialyl Lewis A) for evaluating tumour aggressiveness and prognosis (Yamada et al., 1995)
TSP-1	Expression correlates with independent prognostic factors (Miyanaga et al., 2002)