RUNX3 is believed to have tumour suppressor properties in several cancer types. Inactivation of RUNX3 has been shown to occur by methylation-induced transcriptional silencing and by mislocalization of the protein to the cytoplasm. The aim of this study was to examine the clinical significance of RUNX3 expression in a large series of colorectal cancers using immunohistochemistry and tissue arrays. With advancing tumour stage, expression of RUNX3 in the nucleus decreased, whereas expression restricted to the cytoplasmic compartment increased. Nuclear RUNX3 expression was associated with significantly better patient survival compared to tumours in which the expression of RUNX3 was restricted to the cytoplasm (P = 0.025). These results support a role for RUNX3 as a tumour suppressor in colorectal cancer.

Keywords: RUNX3; colorectal cancer; tissue arrays; prognosis; Wnt
MATERIALS AND METHODS

Tissue microarrays

Sections from tissue microarray (TMA) blocks containing 849 CRC (stages I–IV) and matching normal tissue samples were obtained from the West Australian Research Tissue Network, Department of Radiation Oncology, Sir Charles Gairdner Hospital. Construction of the TMAs and the tumour and patient characteristics have been described elsewhere (Chai et al., 2004). Microsatellite instability (MSI) status was determined using the BAT26 mononucleotide marker as described previously (Chai et al., 2004). Ethics approval to perform this study was obtained from the Human Research Ethics Committee of the Sir Charles Gairdner Hospital.

Immunohistochemistry

Sections from tissue array blocks were cut at 5 μm thickness and stained for RUNX3 by immunohistochemistry using the monoclonal antibody clone R3-6E9 as described previously by our group (Ito et al., 2005). The R3-6E9 clone recognises an epitope within the 191–234 amino-acid region. Its specificity for human RUNX3 has been shown by Western blot analysis and by the removal of immunoreactivity to normal gastric mucosa following preincubation with RUNX3 peptide (Ito et al., 2005). Rehydrated TMA sections were warmed in target retrieval solution (DAKO, Glostrup, Denmark) at 96°C for 40 min, treated with a serum-free blocking solution (DAKO), and then incubated overnight at 4°C with 1 μg/ml R3-6E9 in a diluent solution (DAKO). A peroxidase-3, 3'-diaminobenzidine-based detection system (EnVision + kit, DAKO) was used to detect immunoreactivity. Staining was graded for intensity on a scale of 0–4 in the nuclear and cytoplasmic compartments. The scoring was performed by a single pathologist (NS) following consultation with another pathologist (MST) and in the absence of information on patient outcome or tumour pathology. RUNX3 was considered to be expressed in the nucleus or cytoplasm if the intensity was estimated at ≥2.

RESULTS AND DISCUSSION

Typically observed immunohistochemical staining patterns for RUNX3 are shown in Figure 1. Using a staining intensity of ≥2 as the threshold, RUNX3 expression was expressed in the nucleus of 631/849 (74%) tumours and in the cytoplasm of 431/849 (51%) tumours. Both nuclear and cytoplasmic expressions were present in 352 (41%) tumours, nuclear expression only in 279 (33%), neither nuclear nor cytoplasmic expression in 139 (16%) and cytoplasmic expression only in 79 (9%) tumours. In this study, tumours with ‘any nuclear’ staining were considered to express ‘active’ RUNX3 (n = 631, Group A) (Lau et al., 2006). Tumours with no nuclear or cytoplasmic staining (Group B) or with expression in the cytoplasm only (Group C) were considered to have ‘inactive’ RUNX3.

The associations between RUNX3 expression and clinicopathological and molecular features of the CRC series are shown in Table 1. No significant associations were seen with patient age or gender, or with the histological grade of the tumour. Tumours located in the proximal colon displayed a trend for less frequent expression of nuclear RUNX3 compared to those arising in the distal colon and rectum. A likely explanation for this finding is that CIMP + and RUNX3 methylation are known to occur more often in the proximal colon (Weisenberger et al., 2006) and are associated with methylation-induced transcriptional silencing. The MSI + phenotype is also closely associated with the CIMP + phenotype, thus accounting for the lower frequency of nuclear RUNX3 expression observed in MSI + tumours (Table 1).

In keeping with its postulated role as a tumour suppressor, advanced stage tumours (AJCC stages III and IV) showed less frequent expression of nuclear RUNX3 compared to early stage tumours (Table 1). Kaplan–Meier analysis confirmed that patients with nuclear RUNX3 expression (n = 631, Group A) had significantly better survival outcomes (P = 0.025, logrank test) compared to the relatively small number of patients (n = 79, Group C) in which RUNX3 expression was restricted to the cytoplasm (Figure 2). This result agrees with observations made in oesophageal cancer and is consistent with current understanding of the role of RUNX3 as a tumour suppressor (Sakakura et al.,...
RUNX3 expression has been reported by our group to represent mislocalised and probably inactive protein in gastric and breast cancers (Ito et al., 2005; Lau et al., 2006; Subramaniam et al., 2009). The present results showing that cytoplasmic expression is associated with worse patient outcome support the contention that RUNX3 is in an inactive state.

Interestingly, the subgroup of CRC patients with no or very low expression of RUNX3 in the nucleus or cytoplasm (Group B) showed similar prognosis to those with nuclear RUNX3 expression (Group A, Figure 2). This contrasts with several other cancer types (Araki et al., 2005; Wei et al., 2007; Sakakura et al., 2007) and suggests that the presence or absence of RUNX3 may play a lesser role to its cytoplasmic localisation in determining clinical phenotype in CRC. It should be highlighted, however, that two of the earlier studies did not distinguish between nuclear and cytoplasmatic staining (Araki et al., 2005; Wei et al., 2005).

In summary, the major findings of this study were that nuclear RUNX3 expression was reduced in advanced stages of CRC and that exclusively cytoplasmic expression of RUNX3 was associated with worse patient outcome.

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