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Original Paper

Expression of Neural *BC1* RNA: Induction in Murine Tumours

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***BC1* RNA is a small cytoplasmic RNA polymerase III transcript that is expressed in the rodent nervous system. The RNA is selectively expressed in neurons where it is located in somatodendritic domains. *BC1* RNA is not normally detectable in non-neuronal somatic cells; it is however expressed in germ cells and in cultured immortal cell lines of various non-neural origins. We therefore sought to establish whether the neuron-specific regulation of *BC1* expression is altered in non-neural tumour cells. Oncogen and chemical carcinogen induced mouse tumours were analysed for the presence of *BC1* RNA, using Northern transfer and *in situ* hybridisation. Here we report that *BC1* RNA is selectively expressed in tumour cells, but not in corresponding normal tissues. These results indicate that neural-specific regulation of *BC1* expression is lacking in murine tumour cells of non-neural origin.**

Key words: biological tumour marker, *in situ* hybridisation, regulation of gene expression in tumours, RNA polymerase III transcription

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INTRODUCTION

RODENT *BC1* RNA is a cytoplasmic RNA of 152 nucleotides that is transcribed by RNA polymerase III under cell-type specific regulation [1, 2]. The RNA can be subdivided into three structural domains. The 5′ domain is similar in sequence to the repetitive ID elements (for which the *BC1* gene is the founder gene; see [3]). It is followed by an internal A-rich region and a 3′ sequence domain that is unique to *BC1* RNA [1]. The RNA is transcribed from a single gene present in the genome of rodents ([1]; reviewed in [4]). *BC1* RNA is expressed in neurons of the central and peripheral nervous system, not however in non-neural tissues such as, among others, kidney, liver, lung, spleen, and skeletal and cardiac muscle ([1, 2, 5]; reviewed in [6]). The expression pattern of *BC1* RNA in the nervous system is distinctly heterogeneous and unique. High levels of the RNA have been detected in some grey matter areas, while no significant labelling has been observed in white matter areas [5]. *BC1* RNA has been detected in somatic and/or dendritic domains of a subset of neurons [5] where it is complexed with proteins to form a ribonucleoprotein particle [7, 8]. The neural expression pattern of *BC200* RNA, the primate analogue of *BC1* RNA, has been found to be equivalent to that of *BC1* RNA in all brain areas analysed [9]. Therefore, it has been hypothesised that these RNAs may play equivalent roles, possibly in transport and/or translation of dendritic mRNAs, in somatodendritic domains of neurons in rodents and primates, respectively ([5, 9]; reviewed in [6]).

BC1 RNA is expressed in germ cells such as spermatogonia and oocytes [6], and in cultured immortal cell lines of various non-neural origins [2]. We now report that *BC1* RNA is also expressed in murine tumour tissues: we observed that *BC1* RNA accumulated selectively in tumour cells, yet remained undetectable in corresponding normal tissues.

MATERIALS AND METHODS

Mouse tumour samples were obtained from P. Srivastava (Department of Pharmacology, Mount Sinai School of

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Medicine, New York, U.S.A.). Fibrosarcomas of the skin were induced by methylcholanthracene [10]; adenocarcinomas of the colon were induced by cycasin [11]. Fibrosarcoma cells and adenocarcinoma cells from such animals were cultured and used to produce fibrosarcomas of the skin and adenocarcinomas of the colon, respectively, by local inoculation of host mice. Primary breast carcinomas were directly induced by the *ras* oncogene in transgenic mice [12]; three such mice were analysed in this study.

Preparation and analysis of RNA

Mouse tumour and normal tissues were removed and immediately frozen in liquid nitrogen. Total RNA was isolated by homogenisation in guanidinium thiocyanate and ultracentrifugation through caesium chloride [13]. RNA was fractionated on 1.8% agarose–formaldehyde gels, transferred to GeneScreen membranes (DuPont), immobilised by UV illumination and hybridised to oligonucleotide probe HT005. This probe is complementary to the 3′ unique region of *BC1* RNA and has the sequence:

5′*AAAGGTTGTGTGTGCCAGTTACCTTGTTT*

TTTTTTGGTCTTTTTGTTATTTTGTCTTTTT 3′.

It was end-labelled with [γ-^{32}P]ATP, using T4 polynucleotide kinase. Following hybridisation, the filters were rehybridised to a rat β-actin probe (kindly provided by L. H. Wang, Department of Microbiology, Mount Sinai School of Medicine, New York, U.S.A.). Hybridisation was performed at 42°C for the *BC1* probe, and at 60°C for the rat β-actin probe, in 1 M NaCl, 0.5 M Tris–HCl (pH 7.5), 5× Denhardt's reagent [13], 1% sodium dodecyl sulphate (SDS), 0.1 mg/ml yeast tRNA. The membranes were then washed three times at 55°C in 0.5× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate) and 0.1% SDS for 30 min for the *BC1* probe, and at 65°C in 0.1% SSC and 0.1% SDS for 30 min for the rat β-actin probe.

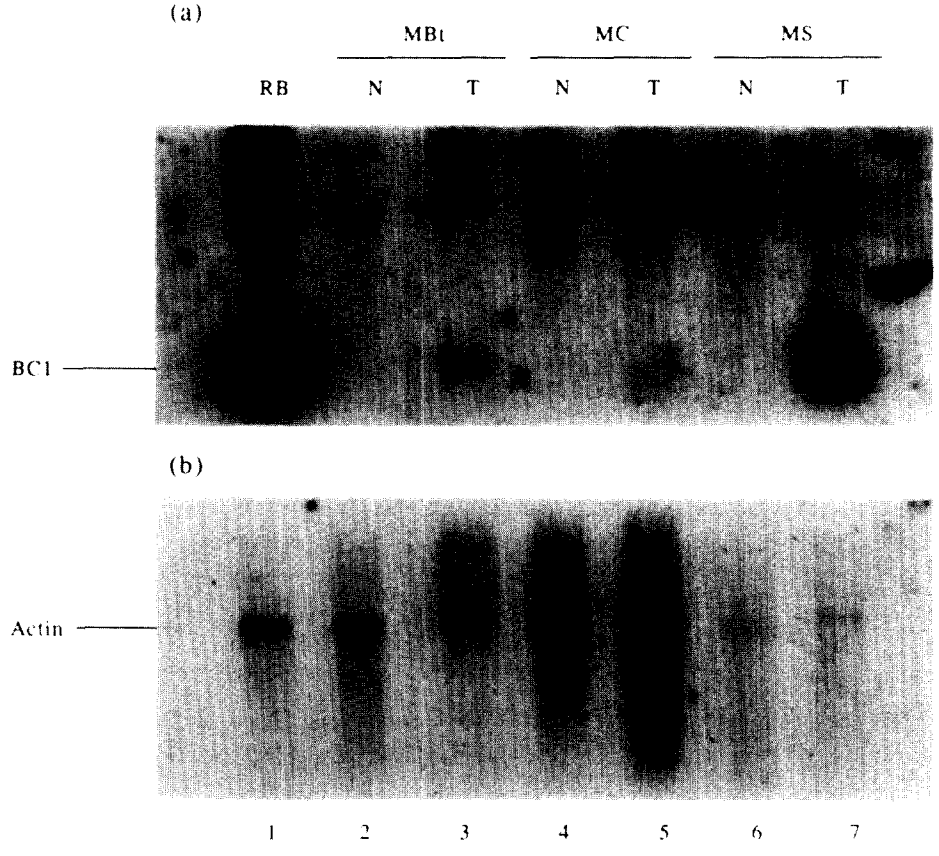

Figure 1. Induction of *BC1* RNA in mouse tumour tissues. (a) Total RNA was extracted from rat brain (positive control) and mouse tumour and normal tissues, as indicated. Ten micrograms were loaded per lane for rat brain, mouse breast and mouse colon samples, 5 μg for mouse skin samples. Different amounts of RNA were loaded in different tissues because levels of *BC1* expression varied between these tissues. However, identical amounts were loaded for each normal and tumour tissue pair. The RNA blot was hybridised to probe HT005 which is complementary to the 3′ unique region of *BC1* RNA. Significant amounts of *BC1* RNA were detected in rat brain (RB) as well as in mouse breast tumour (MBt-T), colon tumour (MC-T) and skin tumour (MS-T), not however in the respective normal tissues (N, normal; T, tumour). In the case of colon and skin, normal tissues were from areas directly adjacent to the tumour tissues. In the case of mammary tissue, the RNA analysed in this experiment was from a tissue sample from a normal (i.e. not *ras* oncogene induced) animal. It should be noted that although all three tumour types were *BC1*-positive in each of the several respective samples analysed, there was some degree of animal-to-animal variation in *BC1* RNA labelling intensities in such tumour tissues. (b) The blot shown in (a) was rehybridised with a β-actin probe. Similar signal intensities were observed in paired tissues (tumour versus normal). In addition, equal loading was ascertained by ethidium bromide staining of ribosomal 18S and 28S RNA bands (not shown).

In situ *hybridisation*

Freshly removed mouse tumour and normal tissues were quick-frozen in liquid nitrogen, embedded in TissueTek OCT embedding medium (Miles), and sectioned in a Bright Microtome Cryostat at 10 μm thickness. Sections were collected on gelatin and poly-L-lysine coated microscope slides.

The probe used to detect mouse *BC1* RNA in *in situ* hybridisation experiments corresponded to the 60 3′-most nucleotides of *BC1* RNA. It was subcloned into the pBluescript KS (+) transcription vector (Stratagene), resulting in plasmid pMK1 [5]. ^{35}S-labelled RNA probes were transcribed from prelinearised templates, using T3 (for sense strand) or T7 (for antisense strand) RNA polymerase.

In situ hybridisation experiments were performed as previously described [14]. This protocol uses UV light as a cross-linking agent to improve signal-to-background ratios.

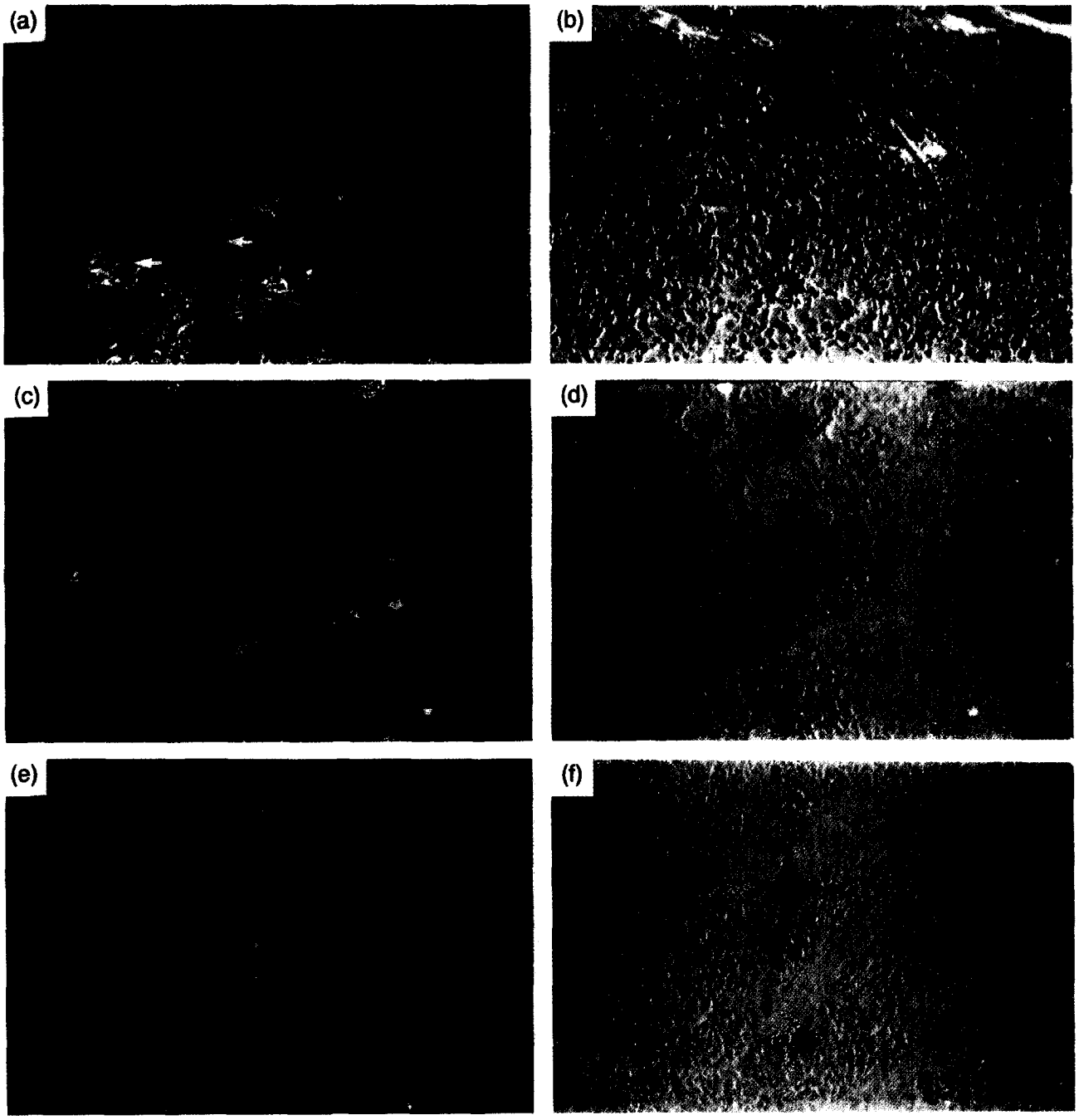

Figure 2. Distribution of *BC1* RNA in mouse skin and colon tumours. (a, b) Mouse skin tumour; (c)–(f) mouse colon tumour. Tissue samples were from the same respective animals as those in the Northern blot experiments (Figure 1). A ^{35}S-labelled probe specific for *BC1* RNA (antisense strand) was used in (a)–(d). A *BC1* RNA sense strand control probe was used in (e, f). A *BC1* RNA labelling signal was observed in most tumour cells (indicated by solid arrows). No significant labelling was detectable in the normal epidermis (including rete ridges; open arrow in (a, b)). Low-level brightness in the epidermis (uppermost tissue layer in (a, b)) is caused by structures in the tissue, not by label-indicating silver grains. Only background labelling was detected with the sense strand control probe (e, f); silver grains are indicated by solid arrows. Darkfield (a, c, e) and DIC Nomarski (b, d, f) optics, Nikon Microphot-FX. Scale bar = 30 μm.

RESULTS

Expression of *BC1* RNA in three different mouse tumours was examined by Northern hybridisation. Total RNA from three different types of tumours and from corresponding normal tissues were hybridised to a ^{32}P-labelled oligonucleotide probe (HT005) which was complementary to the 3′ unique region of *BC1* RNA. RNA from rat brain was used as a positive control. A *BC1* RNA labelling signal was observed in a mouse breast carcinoma, in an adenocarcinoma of the colon and in a fibrosarcoma of the skin (Figure 1a). Significant albeit varying amounts of *BC1* RNA were expressed in these tumour tissues. Among the three types of tumour analysed, highest *BC1* expression levels were observed in the fibrosarcoma of the skin, lowest levels in the adenocarcinoma of the colon, and intermediate levels in the mouse breast carcinoma. *BC1* RNA was not detected in the corresponding normal tissues (Figure 1a). The same blots were rehybridised to a β-actin probe. This probe, which is specific for a ubiquitously expressed mRNA, was used as a control to monitor the quantity and integrity of the RNA on the filter (Figure 1b). Similar signal intensities were observed in the paired tissues. These data indicate that the neuron-specific control of *BC1* expression was lost in the tumours analysed here.

We further analysed the distribution of *BC1* RNA in mouse tumour tissues by *in situ* hybridisation (Figures 2 and 3). In a cutaneous fibrosarcoma, the normal morphology of the dermis was no longer recognisable, whereas the epidermis was still intact (Figure 2a, b). Pleomorphic fibroblasts were widely distributed in the dermis. A strong *BC1* RNA labelling signal was observed in most, if not all, neoplastic cells, with no significant labelling in the normal epidermis, including the rete ridges (Figure 2a, b). Figures 2c and d show *BC1* RNA labelling in a mouse colon adenocarcinoma. The normal morphology of the colon was destroyed, the tissue being displaced by a poorly differentiated adenocarcinoma. *BC1* RNA was again expressed by most, if not all, tumour cells. The sense strand control probe failed to produce specific labelling in any of the examined tissues (Figure 2e, f and data not shown). Figure 3 shows the expression of *BC1* RNA in a murine breast carcinoma induced by the *ras* oncogene. Whereas no significant *BC1* labelling was detectable in normal mouse breast tissue, *BC1* RNA was detected in some, but not all, areas of the tumour tis-

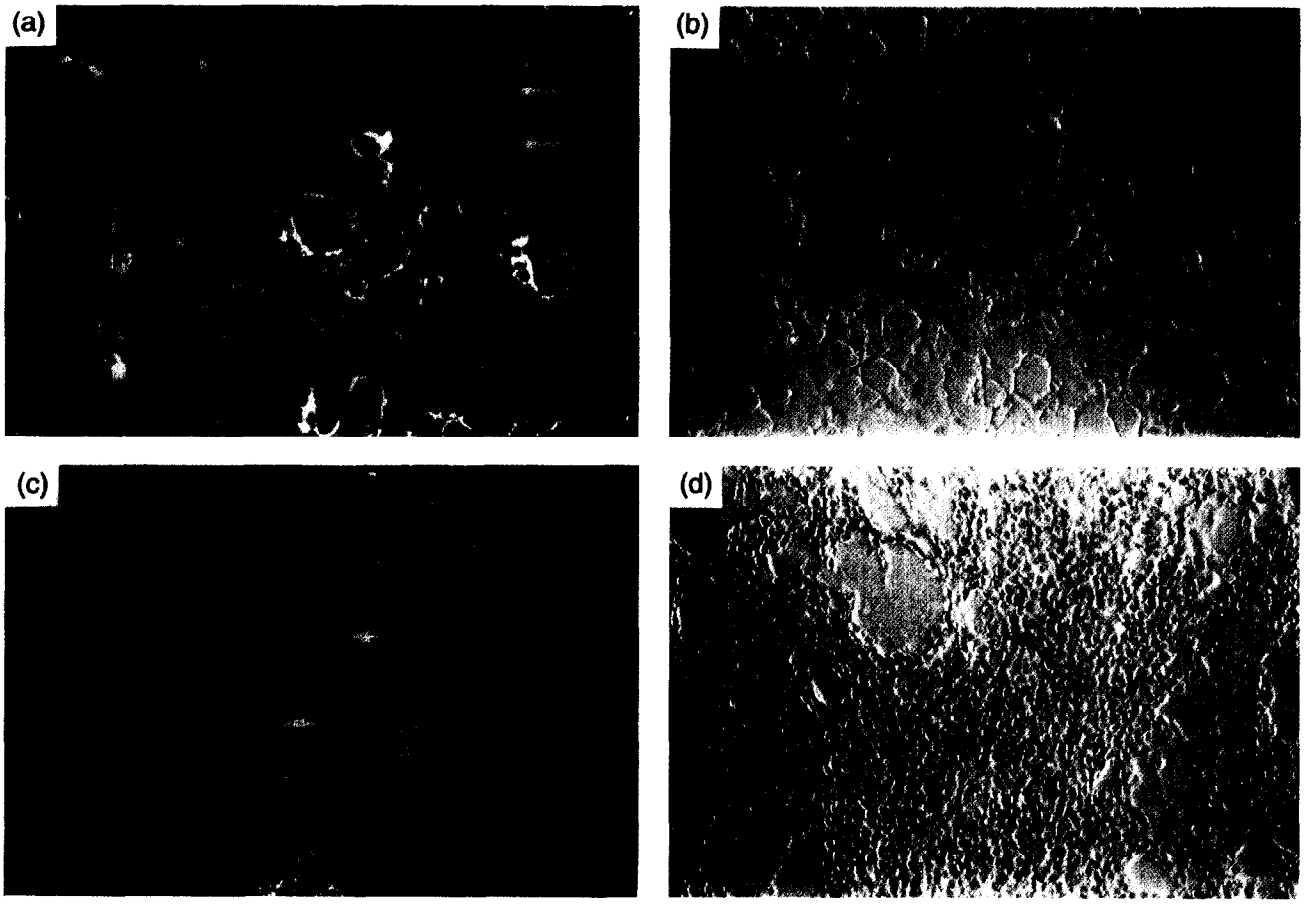

Figure 3. Distribution of *BC1* RNA in a mouse breast tumour. (a, b) Normal breast tissue; (c, d) breast tumour tissue; normal and tumour tissues were from two different animals. A *BC1* RNA labelling signal was observed in some areas of the tumour (solid arrows in (c) and (d)), but not in others (open arrows in (c) and (d)). No significant *BC1* signal was detectable in normal breast tissue (a, b); arrows in (a) indicate background silver grains. The brightness that appears in circular structures in the darkfield photomicrograph of normal breast tissue (a) is caused by fat-containing tissue. In control experiments with a sense strand probe, background labelling was observed that was similar to that seen in Figure 2 (e, f) (not shown). Darkfield (a, c) and DIC Nomarski (b, d) optics, Nikon Microphot-FX. Scale bar = 30 μm.

sue. The labelling pattern often varied among different regions within the same section; areas with extensive *BC1* labelling of tumour cells were found adjacent to areas where tumour cells showed no significant labelling (Figure 3). The significance of this observation remains to be established. *BC1*-negative tumour cells may, for example, represent such cells that have progressed towards a more advanced state of differentiation, on a path that would ultimately result in their death and elimination. Nevertheless, non-tumour cells were unlabelled, and *in situ* hybridisation studies thus confirmed that *BC1* expression was restricted to tumour cells and was not detectable in normal cells.

DISCUSSION

In this paper, we have demonstrated that the neural specificity of *BC1* RNA gene expression was lost in a *ras* oncogene and in chemical carcinogen induced mouse tumours. *BC1* RNA expression was observed in tumour cells, but not in corresponding normal tissues. Since the fibrosarcomas of the skin and adenocarcinomas of the colon were induced by local inoculation with cells from a methylcholanthrene-induced fibrosarcoma line and a cycasin-induced adenocarcinoma line, respectively, it is possible that the positive *BC1* signal in such cases is due to the propagation of tumour cells that were *BC1*-positive prior to inoculation. For this reason, we decided to include an oncogene-induced primary tumour in our analysis. Transgenic mice have been used as models for the molecular analysis of carcinogenesis for almost a decade. Overexpression of oncogenes, such as *ras*, *myc* and *neu*, in mammary tissue, results in the development of breast cancers [15]. The mouse breast tumours that were used in our studies were induced by the *ras* oncogene. The identification of *BC1* RNA in such tumours thus confirms that the neural-specific regulation of *BC1* expression is lacking in neoplastic cells in primary tumours, and it further indicates that *BC1* expression may be a consequence or causal correlate of tumour induction and/or progression.

BC1 RNA is a neural-specific RNA polymerase III transcript (reviewed in [6]). In addition to general polymerase III transcription factors (such as TFIIIB and TFIIIC), the presence in neurons of specific protein activators, or the absence of silencer binding proteins (for reviews, see [16, 17]), may contribute to the neuron-specific expression of *BC1* RNA. It is thus possible that during tumorigenesis, such factors could be over- or underexpressed, respectively, in tumour cells. Alternatively or in addition, the stability of the RNA may be enhanced by other factors. It should also be noted that earlier work with transformed cells has demonstrated a stimulation of transcription by RNA polymerase III for repetitive elements such as rodent B2, but not for small stable RNAs such as 5S ribosomal RNA or tRNAs [18].

Our recent studies have shown that *BC200* RNA, the primate *BC1* analogue, was also expressed in many, but not all, human primary tumours (W.C., W. Böcker, J.B. and H.T., University of Münster, Germany). High levels of *BC200* labelling were observed in most neoplastic cells in *BC200*-positive tumours, with no significant staining in the adjacent normal tissues. The labelling pattern of *BC1* RNA and *BC200* RNA in tumour tissues was very similar in that malignant cells were the exclusive sites of expression for either RNA. It is therefore possible that loss of tissue-specificity of *BC1* and *BC200* expression occurs during tumorigenesis via similar or identical mechanisms. While the functional role of *BC1* RNA in the pathogenesis of tumours remains an open question, it is hoped that the causal interrelation between *BC1* expression and the induction and/or progression of neoplasia can be elucidated in future studies.

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