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Bcl-2 protein localizes to the chromosomes of mitotic nuclei and is correlated with the cell cycle in cultured epithelial cell lines

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SUMMARY

***bcl*-2 gene expression confers a survival advantage by preventing cells from entering apoptosis. In contrast to the previously described cytoplasmic localization of Bcl-2 in epithelial cells in vivo, in this study we have demonstrated, in a series of human epithelial cell lines, that Bcl-2 also localizes to mitotic nuclei. Both immunocytochemical and immunoelectron microscopical examinations localize this protein to nuclei and in particular to chromosomes. Nuclear Bcl-2 expression in these cell lines is correlated with the cell cycle. There is relatively strong expression during mitosis, most intense during prophase and metaphase, declining in telophase and then the protein becomes undetectable soon after separation of the two daughter cells. The expression and distribution of Bcl-2 is influenced by treatment with excessive thymidine. These results indicate that Bcl-2 may protect the cells from apoptosis occurring during mitosis and suggest a possible role for the protein in cell immortalization.**

Key words: *bcl*-2, cell cycle, proliferation, epithelial cells

INTRODUCTION

The B cell leukaemia/lymphoma gene 2 (*bcl*-2) was discovered in the study of the t(14;18) chromosome translocation present in B cell leukaemia and a high proportion of follicular lymphomas (Yunis et al., 1982; Tsujimoto et al., 1984; Cleary et al., 1986). Subsequent studies have reported *bcl*-2 expression in a wide range of lymphoproliferative diseases that lack this chromosomal abnormality (Pezzella et al., 1990; Zutter et al., 1991), and in a variety of normal adult and embryonic tissues, including epithelial and mesenchymal tissues (Hockenbery et al., 1991; Lu et al., 1993a).

Bcl-2 protein has been reported to be associated with intracellular membranes, predominantly localized to the perinuclear endoplasmic reticulum or to mitochondrial membranes (Chen-Levy et al., 1989, 1990; Hockenbery et al., 1990, 1993; Monaghan et al., 1992). Bcl-2 confers a survival advantage to lymphoid cells, fibroblasts and other types of cells by inhibiting apoptosis (mAb., 1989; Nunez et al., 1990; Hockenbery et al., 1990; Bissonnette et al., 1992; Fanidi et al., 1992). This protective effect has been suggested to be important in the development of the immune system (Nunez et al., 1991; McCarthy et al., 1992), cell maturation and differentiation (Schena et al., 1992; Lu et al., 1993b), virus-induced B cell immortalization (Tsujimoto, 1989; Gregory et al., 1991), and in the development of lymphoid malignancies (Reed et al., 1988). The *bcl*-2 gene may also be involved in cell transformation in co-operation with the c-*myc* gene in vitro (Vaux et al., 1988; Fanidi et al., 1992). These data suggest that *bcl*-2 gene activation may play a role in cell immortalization and the multistep evolution of malignancy.

In this study, we have examined a number of epithelial cell lines for the expression of the *bcl*-2 gene to gain insight into what role, if any, the protein plays in the immortalization process. In particular we have examined the expression of Bcl-2 protein during the cell cycle. The results show, for the first time, that Bcl-2 localizes to mitotic nuclei, and specifically to chromosomes and that the strength of expression varies within the cell cycle.

MATERIALS AND METHODS

Cell lines

Origin and culture

Nine epithelial cell lines (from the Cell Production Department, Imperial Cancer Research Fund, UK) were investigated for Bcl-2 expression (see Table 1). MTSV1-7 was cultured in Dulbecco's modified Eagle's medium (DMEM) with insulin (10 μg ml^{-1}; Sigma, UK), hydrocortisone (0.5 μg ml^{-1}; Calbiochem, UK) and 10% foetal calf serum (FCS; Bartek et al., 1991). BT20 was cultured in DMEM, insulin (10 μg ml^{-1}) and 10% FCS. The others were grown in DMEM and 10% FCS alone (referred to as normal medium).

Cell cycle arrest

To examine the association of Bcl-2 protein expression with the cell cycle, cells from T47D and MTSV1-7 were cultured to 50% confluence and then subjected to a range of blocking methods. Cells were arrested in the G_o phase by serum deprivation (without FCS), in the late G_1 phase by isoleucine deprivation (isoleucine-free DMEM with 10% dialyzed and stripped FCS), and in the S phase by thymidine excess (10% stripped FCS and 2 mM thymidine; Sigma) for 48 hours. Cells were also arrested in metaphase by colcemid (20 ng ml^{-1}; Sigma) and vincristine (1 μg ml^{-1}; Sigma) treatment for 4 hours. In order to assess the efficiency of the cycle blockage the cells were subsequently incubated with bromo-deoxyuridine (BrdU) for 2 hours using the Cell Proliferation Kit (Amersham, UK) and the incorporation was detected with mouse anti-BrdU antibody and peroxidase-conjugated rabbit anti-mouse immunoglobulin (Ig).

Preparation for immunocytochemical staining

Cells were cultured in 35 mm Nunc (Denmark) Petri dishes to about 80% confluence and then washed with phosphate-buffered saline (PBS), air-dried and fixed with methanol for 10 minutes at room temperature (RT). In order to assess the staining of chromosomes mitotic cells were removed from the dishes 4 hours after the addition of colcemid (20 ng ml^{-1}). These cells were washed with PBS and smears were made by dropping a concentrated preparation onto glass slides. In addition, cell pellets were prepared, fixed in neutral buffered formalin and processed as cell blocks (in paraffin wax), from which sections were cut.

Antibodies and immunocytochemistry

Paraffin sections were microwaved at 700 watts in 0.01 M tri-sodium citrate, pH 6.0, for 10 minutes. Cell preparations and sections were incubated with the following antibodies: (i) two monoclonal antibodies (mAbs) bcl-2/100 and bcl-2/124 (both IgG1) raised against a synthetic peptide corresponding to amino acids 41-54 of Bcl-2 protein (bcl-2/124, 1:10 (Dakopatts, Denmark); bcl-2/100 1:1 (a gift from Dr D. Y. Mason, Oxford, UK)) in 3% bovine serum albumin (BSA) in PBS for 60 minutes followed by rabbit anti-mouse Ig (1:20; Dakopatts) and mouse peroxidase anti-peroxidase (mPAP, 1:100; Dakopatts) for 60 minutes, respectively. (ii) A polyclonal rabbit-antibody, Bcl-2/1631-13, raised against the same synthetic peptide of Bcl-2 protein. This antibody was detected by swine anti-rabbit Ig (1:100; Dakopatts) and rabbit peroxidase anti-peroxidase (rPAP, 1:100; Dakopatts).

Peroxidase activity was developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma) and hydrogen peroxide and samples were counterstained with haematoxylin.

Incubation of the primary Bcl-2 antibodies with the synthetic immunising peptide (2 mg ml^{-1}) for 30 minutes abrogated staining of known positive controls. Incubation without primary antibody was used as a control to assess endogenous peroxidase activity.

Frozen and neutral buffered formalin-fixed, paraffin-embedded human tonsils were used as positive tissue controls.

Immunoelectron microscopic localization

Cell lines MTSV1-7, T47D and PANC-V were fixed with 3% paraformaldehyde and 0.05% monomeric glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.4) for 15 minutes at RT. After fixation the cells were treated with 50 mM glycine in phosphate buffer for 15 minutes and washed in the buffer at 4°C. The cells were dehydrated in a graded series of methanols at progressively lower temperatures and infiltrated with Lowicryl HM 20 resin at −50°C. The resin was polymerized by UV light for 48 hours at −50°C. Ultrathin sections were mounted on carbon-coated grids and labelled as follows. After preincubation with 5% normal goat serum, 5% ovalbumin and 5% BSA in PBS for 60 minutes, the grids were transferred onto drops of bcl-2/100 antibody (neat hybridoma supernatant) and incubated overnight at 4°C. The sections were then washed with PBS followed by incubation on drops of gold (1 nm)-conjugated goat anti-mouse IgG for 2 hours. After washing with PBS and distilled water (15 minutes each), silver enhancement of the gold labelling was carried out for 4 minutes at RT using Intensive M Silver Enhancement Kit (Amersham). The sections were then washed, air-dried, contrasted with uranyl acetate and examined with a Jeol 1200 FX electron microscope at 80 kV. Sections without primary antibody incubation were used as negative controls.

Immunoblotting

Protein preparations were made from the cell lines HT29, T47D and MTSV 1-7, grown to approximately 80% confluence in normal medium. For enrichment of mitotic cells, cells were shaken off the dishes and collected 4 hours after the addition of colcemid (20 ng ml^{-1}). A whole cell population was also collected. After staining with Giemsa the proportion of cells in mitosis was counted per 1000 cells. There were 38%, 41% and 27% mitotic cells in mitotic cell-enriched populations and 2%, 3% and 2% in whole cell populations of HT29, T47D and MTSV1-7, respectively. The same number of cells (10^6 cells) was lysed in 500 μl of RIPA buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 0.5% sodium deoxycholate, 0.5% NP-40, 0.1% SDS and 1 mM phenylmethylsulfonyl fluoride). Sample DNA was sheared through a 26-gauge needle, boiled for 5 minutes and stored in aliquots at −20°C prior to use. For SDS-polyacrylamide gel electrophoresis, samples were boiled for 8 minutes in an equal volume of sample buffer (0.02% bromophenol blue, 4% SDS, 4% 2-mercaptoethanol, 20% glycerol and 50 mM Tris-HCl) and loaded on 12% gels. Similar amount of protein loading was confirmed by Coomasie Blue staining. Western blots of proteins on nitrocellulose (Amersham) were analysed for Bcl-2 protein with a pool of mAbs bcl-2/100 and bcl-2/124 (1:20 dilution). Proteins were detected with biotinylated rabbit anti-mouse Ig (1:1000; Dako), peroxidase-conjugated streptavidin (1:2000; Dako) and developed with DAB and hydrogen peroxide. Protein preparations from the known Bcl-2-positive B lymphocyte cell line T7S-26 were used as controls (Lu et al., 1993a).

RESULTS

Detection of Bcl-2 expression by immunocytochemistry

Positive staining for Bcl-2 protein was observed in all the cell lines examined and was similar with the antibodies used. The staining could be divided into three patterns: cytoplasmic, interphase nuclear and mitotic figure staining (Fig. 1A-C). Clear cytoplasmic staining was only seen in a few cells in the cell lines MCF7 and ME180 and the staining was weak in most positive cells. Interphase nuclear staining, either punctate or diffuse, was seen in all the cell lines examined. Immunoreactivity was also observed in the nucleolar areas (Fig. 1C). Both the intensity of nuclear staining and number of positive cells varied greatly.

The strongest nuclear staining was located in the mitotic cells, most of which contained immunodemonstrable Bcl-2 (ranging from 82 to 97% of mitotic cells in the lines examined; Fig. 1A and Table 1). This staining was almost entirely localized to chromosomes rather than in the nucleoplasm and was best seen on smear preparations prepared from mitotic cell-enriched cell fraction (Fig. 1B). Variation of staining intensity was observed throughout the cell-cycle. The staining was strongest during prophase to metaphase, declining to weak during anaphase (Fig. 1C) and largely absent in telophase when

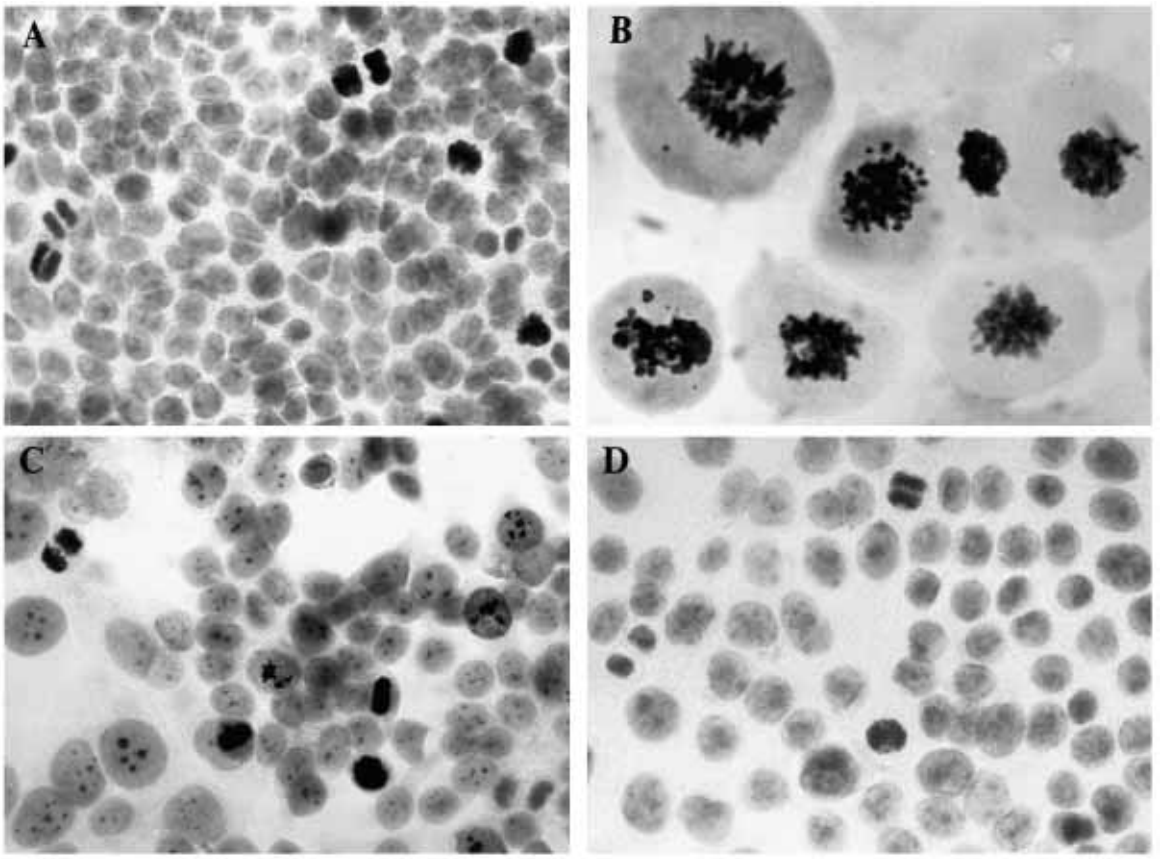

Fig. 1. Immunocytochemical detection of Bcl-2 protein in (A) colon carcinoma cell line HT29; (B) immortalized mammary epithelial cell line MTSV1-7 (cell preparation of the mitotic cell-enriched population); and (C) breast carcinoma cell line MCF7 with mAb bcl-2/100. Nuclear staining for Bcl-2 protein is seen in the mitotic cells. Among these positive cells, stronger staining is seen in prophase and metaphase whereas weaker staining is observed at anaphase (A and C). Positive staining is also present in nucleolar areas with variation in intensity (C). Strong staining is localized to chromosomes whereas cytoplasm is only weakly stained (B). Staining for Bcl-2 is blocked after prior incubation of the antibody with the synthetic peptide (D) (same cell line MCF7 as C).

the two daughter cells were clearly separated. Weak cytoplasmic staining was also seen in some of the positive mitotic cells.

No significant background staining was observed and omission of the primary antibodies resulted in no staining. All staining was blocked after prior incubation of the antibodies with the synthetic peptide (Fig. 1D). In control sections of normal tonsil most lymphocytes in follicular mantle zone were strongly and appropriately stained whereas the majority of follicular center cells were negative.

Correlation of Bcl-2 protein expression with cell cycle

T47D cells cultured in serum-free medium or blocked in G_1 phase by isoleucine starvation for 48 hours showed no mitotic

Table 1. Bcl-2 expression in epithelial cell lines

Cell line	Cell origin	Number of mitoses counted	Bcl-2 positive	Bcl-2* negative	Cytoplasmic† staining
MTSV1-7	SV40-immortalized normal mammary epithelial cells	568	528 (93%)	40	–
MTSV1-7 ras	v-H-Ras-transformed MTSV1-7	544	523 (86%)	21	–
T47D	Breast carcinoma	512	449 (88%)	63	–
MCF7	Breast carcinoma	510	416 (82%)	94	+F
BT20	Breast carcinoma	538	445 (83%)	93	–
PANC -V	Pancreatic carcinoma	550	477 (87%)	73	–
ME180	Cervical carcinoma	528	433 (82%)	95	+F
HT29	Colon carcinoma	524	455 (87%)	69	–
LOVO	Colon carcinoma	501	488 (97%)	13	–

*Most Bcl-2 negative mitotic cells are those at the late anaphase and telophase.
†Cytoplasmic staining in interphase cells. –, no staining for Bcl-2 is clearly observed. +F, a few cells with weak positive staining.

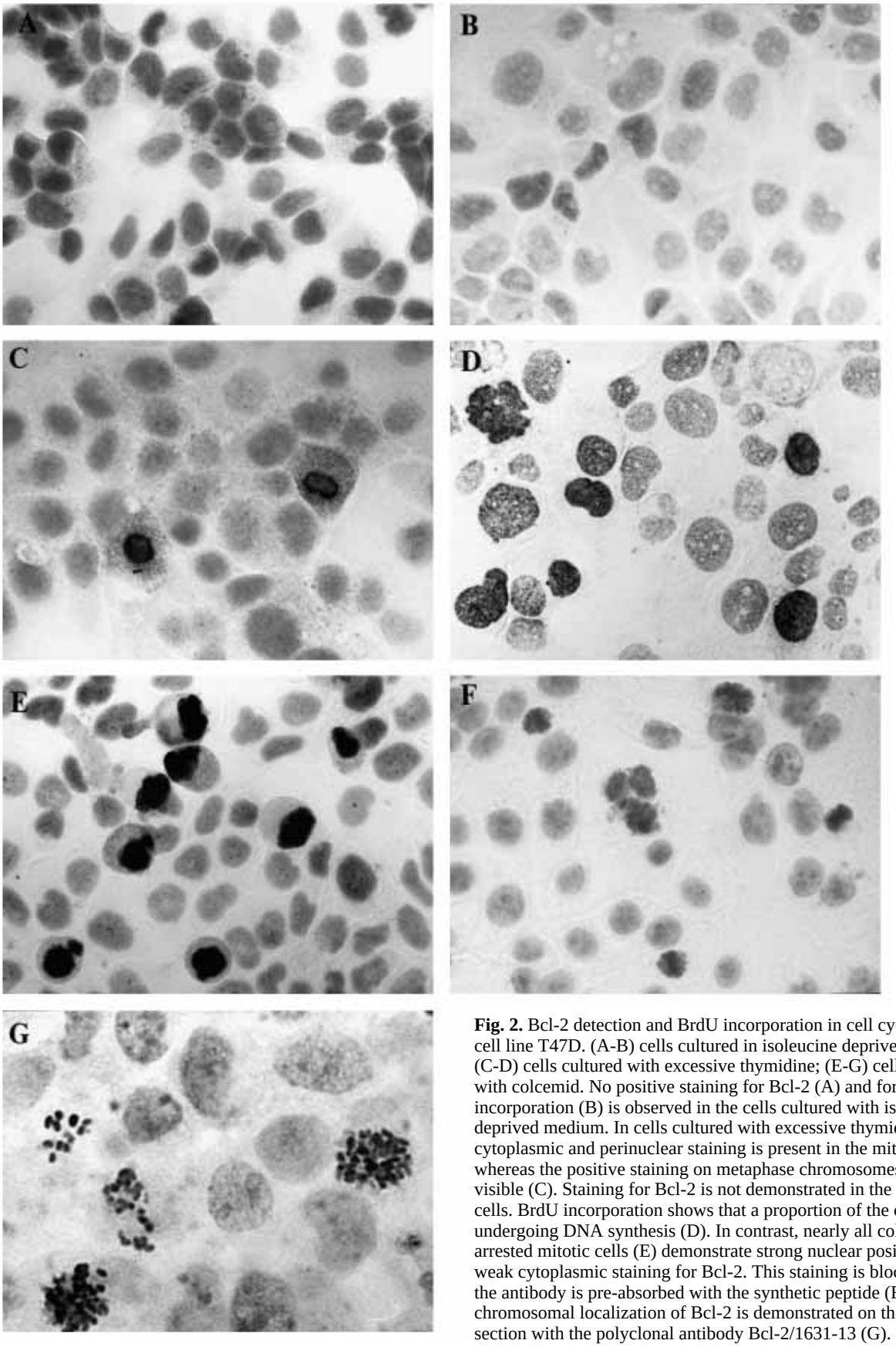

Fig. 2. Bcl-2 detection and BrdU incorporation in cell cycle-arrested cell line T47D. (A-B) cells cultured in isoleucine deprived medium; (C-D) cells cultured with excessive thymidine; (E-G) cells treated with colcemid. No positive staining for Bcl-2 (A) and for BrdU incorporation (B) is observed in the cells cultured with isoleucine-deprived medium. In cells cultured with excessive thymidine, weak cytoplasmic and perinuclear staining is present in the mitotic cells whereas the positive staining on metaphase chromosomes is barely visible (C). Staining for Bcl-2 is not demonstrated in the interphase cells. BrdU incorporation shows that a proportion of the cells are undergoing DNA synthesis (D). In contrast, nearly all colcemid-arrested mitotic cells (E) demonstrate strong nuclear positivity with weak cytoplasmic staining for Bcl-2. This staining is blocked when the antibody is pre-absorbed with the synthetic peptide (F). Clear chromosomal localization of Bcl-2 is demonstrated on the paraffin section with the polyclonal antibody Bcl-2/1631-13 (G). mAb bcl-2/100 is used unless stated otherwise.

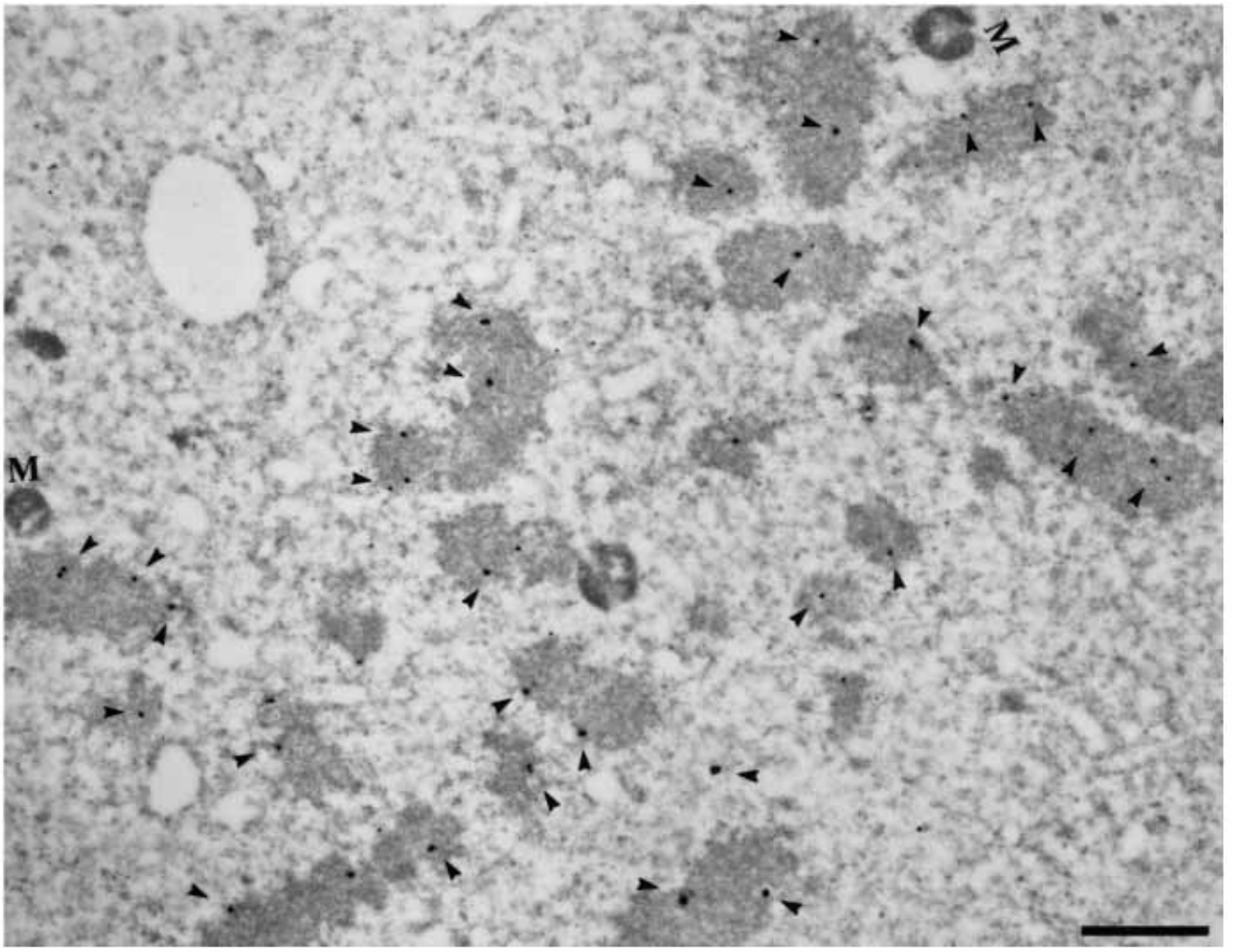

Fig. 3. Bcl-2 protein localization on chromosomes of pancreatic carcinoma cell line PANC-V using immunoelectron microscopy with mAb bcl-2/100. Gold-silver particles are localized at the chromosomes (arrow heads) in a regularly spaced fashion. No labels are observed in the mitochondria (M) or cytoplasm. Bar, 1 μm.

cells and all cells were unstained by the antibodies to Bcl-2 (Fig. 2A). No DNA synthesis was demonstrated by BrdU incorporation (Fig. 2B). After having grown for 48 hours with an excess of thymidine mitotic figures were still seen, indicating an incomplete block of cells in S phase, which was confirmed by BrdU incorporation showing that about 40% of cells were stained (Fig. 2D). Although weak cytoplasmic and perinuclear staining was observed in the mitotic cells, the chromosomes of these cells, however, were mainly unstained by the anti-Bcl-2 antibodies (Fig. 2C), in contrast to the strong chromosome staining observed in cells grown in normal medium. More than 90% of mitotic cell nuclei were strongly positive for Bcl-2 when mitosis was arrested by vincristine or colcemid (Fig. 2E). Cytoplasmic staining, although clearly weaker, was present in most of these nuclear-positive cells. The positive staining was also blocked by pre-absorption with the synthetic peptide (Fig. 2F). The chromosome localization of Bcl-2 protein was clearly demonstrated on paraffin sections with the rabbit anti-human Bcl-2 antibody (Fig. 2G).

Similar results were observed in cell arrest experiments utilising the cell line MTSV1-7.

Chromosome localization of Bcl-2 protein by immunoelectron microscopy

In the cell lines MTSV1-7, T47D and PANC-V, the labelling density for Bcl-2 protein seen with electron microscopy was low compared to the intensity of immunostaining seen by light microscope. The amount and the localization of positive gold-silver particles varied from cell to cell. The strongest labels were observed on condensed chromatin in prophase and on chromosomes in metaphase and early anaphase. The positive labels along the chromosomes appeared to be distributed in a regularly spaced manner (Fig. 3). In early prophase, the labels were localized to the condensed chromatin, but were not associated with the nuclear membrane (Fig. 4). Labels were also seen in association with the membrane of the endoplasmic reticulum (Fig. 5). Little labelling was seen in interphase nuclei and mitochondria. Similar results were observed in all the three

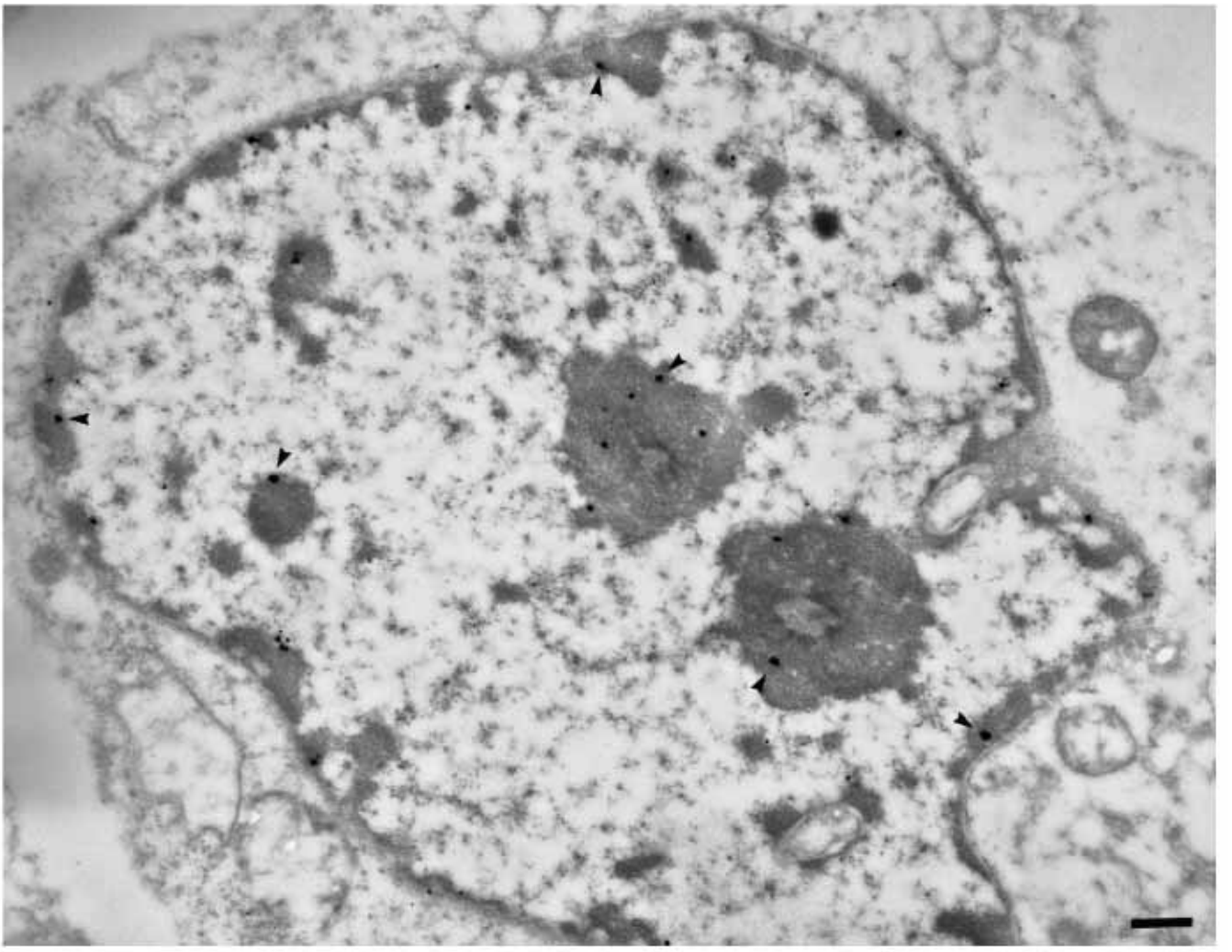

Fig. 4. Bcl-2 protein localization in early prophase of a MTSV1-7 cell using immunoelectron microscopy with mAb bcl-2/100. The labels for Bcl-2 protein are seen on condensed chromatin within the nucleus and abutting the nuclear membrane (arrowheads), but not on the nuclear membrane itself. Bar, 0.5 μm.

cell lines so examined. This type of label distribution was unique and no grains were seen in control sections.

Identification of Bcl-2 protein by immunoblotting

Bcl-2 protein was detected in the two cell lines MTSV1-7 and T47D when the whole cell populations were examined. The weak, but visible positive bands showed M_r of 26 kDa, identical to that observed in the known Bcl-2 protein-positive B-cell line T7S-26 (Fig. 6). Equal amounts of protein extracted from the mitotic cell-enriched population demonstrated an even stronger positivity but similarly located bands. Bcl-2 was also detected from the mitotic cell-enriched population of the cell line HT29 although it was unable to be demonstrated in the whole cell population.

DISCUSSION

Our results demonstrate a new and novel pattern of Bcl-2 expression and show for the first time, by both immunocytochemistry and immunoelectron-microscopy, that the Bcl-2 protein localizes to the nucleus and specifically to chromosomes.

Bcl-2 overexpression provides a survival advantage to various types of cells in vitro (Tsujimoto, 1989; Alnemri et al., 1992; Deng et al., 1993); however, the molecular mechanisms are not yet clearly understood and the subcellular location of the protein has been controversial. Bcl-2 protein does not have a signal peptide, but has a relatively hydrophobic carboxyterminal region (Tsujimoto et al., 1987). Chen-Levy et al. (1990) reported that Bcl-2 protein was tightly associated with microsomal membranes through hydrophobic interaction. By using immunolocalization, Hockenbery et al. (1990) reported that Bcl-2 was located at inner mitochondrial membranes. This group suggested that the main metabolic function of inner mitochondrial membranes may be involved in the regulation of cell survival pathways and that Bcl-2 protects cell from apoptosis by altering mitochondrial function. Other studies

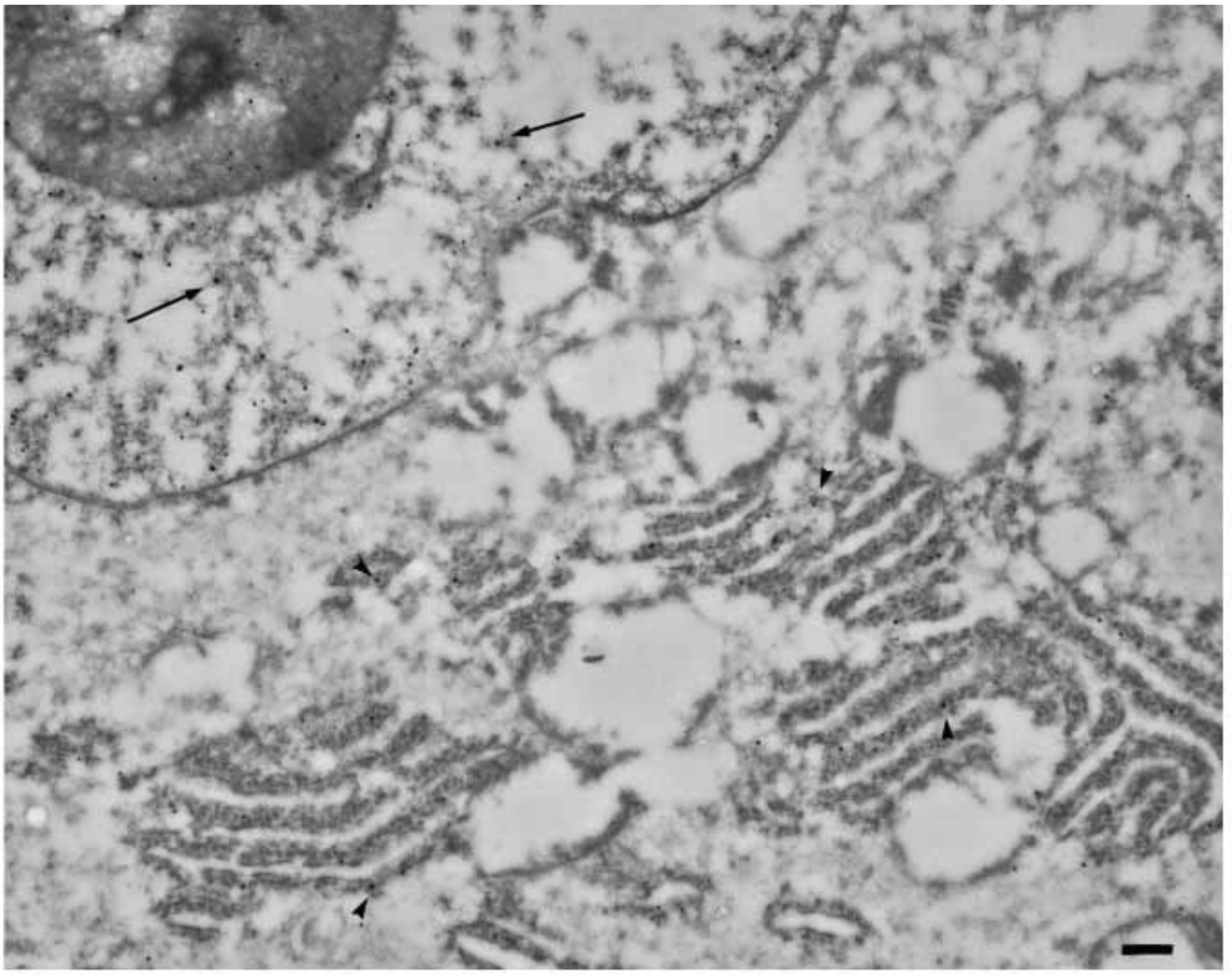

Fig. 5. Bcl-2 protein localization to endoplasmic reticulum (ER) of MTSV1-7 cell using immunoelectron microscopy with mAb bcl-2/100. Gold-silver particles are seen on ER (arrowheads) and in the cell nucleus. Labels in the nucleus are mainly localized over the dense chromatin (arrows), indicating that the cell is at early prophase. Bar, 0.5 μm.

using biochemical fractionation and immunofluorescence analyses, however, showed that Bcl-2 was also associated with nuclear envelope and endoplasmic reticulum (Chen-Levy et al., 1989; Hockenbery et al., 1993) and that its expression blocked apoptosis in cells without mitochondrial DNA (Jacobson et al., 1993). Immunoelectron microscopic study of both lymphoid and epithelial cells showed that Bcl-2 protein was localized throughout cytoplasm and to intracellular membranes including outer mitochondrial membrane (Monaghan et al., 1992). These disparate results, together with our observations, suggest that different mechanisms may be involved in the Bcl-2-mediated cell protection against apoptosis and the location of the protein may change depending on its functional destination.

The nuclear localization of Bcl-2 protein in the cultured cells in our study correlated with the cell cycle, with strong expression during mitosis, a view supported by the clearer demonstration of Bcl-2 protein in cell fractions enriched with mitotic cells when compared with the whole cell populations. Mitosis-associated Bcl-2 expression in these epithelial cell lines appeared independent of cell origin, since immortalized normal mammary epithelial cells and cell lines from a range of carcinoma types all exhibit the same pattern of expression, suggesting that Bcl-2 expression is a general feature in association with replication in these epithelial cell lines. The variation in the expression of Bcl-2 protein during mitosis, with strongest expression in prophase and metaphase, raises some important questions concerning the biological function of this protooncogene. As Bcl-2 protein is known to protect cells from apoptosis induced by growth factor deprivation (Tsujimoto, 1989; Alnemri et al., 1992) or applied stress such as heat shock and ethanol treatment (Deng et al., 1993), it is possible that mitosis-associated Bcl-2 expression has a protective effect on a stage of specific vulnerability. There has been accumulating evidence suggesting that *bcl*-2 is involved in cellular proliferation. Reed et al. (1987) showed the level of *bcl*-2 mRNA of peripheral blood lymphocytes increases within 14 hours after PHA stimulation. A high concordance in the

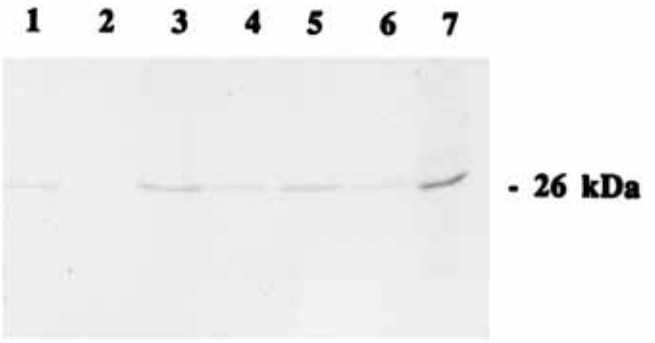

Fig. 6. Immunoblotting for Bcl-2 protein with pooled mAbs bcl-2/100 and bcl-2/124. Lanes 1, 3 and 5 are proteins extracted from mitotic cell-enriched populations of HT29, T47D and MTSV1-7 cell lines, respectively; lanes 2, 4 and 6 are proteins extracted from whole cell populations of HT29, T47D and MTSV1-7 cell lines, respectively; lane 7 is proteins extracted from a Bcl-2-positive B cell line T7S-26. The apparent M_r of the bands for Bcl-2 in the epithelial cell lines is 26 kDa, exactly the same as that observed in T7S-26 (lane 7). Bcl-2 is undetectable in HT29 when protein is extracted from whole cell populations (lane 2). Proteins extracted from the mitotic cell-enriched fractions show positive bands for Bcl-2 stronger than those from whole cell populations.

levels of Bcl-2 and Ki67 antigen (a cell proliferation antigen) coexpression was found in some B cells (Schena et al., 1992) and Bcl-2 expression can partially substitute the requirement of the growth factors for initiation of DNA synthesis in 3T3 fibroblasts (Reed et al., 1991). The use of antisense oligonucleotides to *bcl*-2 mRNA demonstrated growth suppression of a leukemia cell line (Reed et al., 1990). Moreover, *bcl*-2 expression blocked c-*myc*-induced apoptosis not only at G_o phase, but also at S and G_2 phases (Fanidi et al., 1992). Our demonstration of mitosis-associated Bcl-2 expression provides additional and direct evidence for association of *bcl*-2 gene product with the process of cell proliferation. High levels of nuclear Bcl-2 expression may reflect a greater demand for protection of the cells in the mitotic stage, since the genomic DNA in this period is exposed to a new environment, and is therefore potentially vulnerable to endonucleolytic cleavage (Hillion et al., 1991; Compton, 1992). From this evidence we infer that Bcl-2 expression might be involved in promotion of cell survival in vitro through its effects in the cell cycle. This may also explain the absence of Bcl-2 protein on chromosomes when cells are cultured with excessive thymidine, which can severely affect the process of normal DNA replication and cause cell death.

Chromosomal staining for Bcl-2 protein has not been reported previously in vivo. When a series of nasopharyngeal and breast carcinomas as well as normal adult and embryonic tissues were examined, we failed to detect mitosis-associated Bcl-2 expression in these tissues although perinuclear staining was observed in some of the Bcl-2-positive cells (Lu et al., 1993a,b; Chan et al., unpublished results).

The observation of Bcl-2 in mitotic nuclei in vitro systems is unlikely to be due to staining artefacts, since the same tissue fixation (with methanol for fresh tissue and cell preparations and neutral buffered formalin for paraffin blocks), antibodies and detection methods (three step mPAP method) have been used as in our studies cited above. The antibodies employed in this study have been widely used (Pezzella et al., 1990; Monaghan et al., 1992; Lu et al., 1993a) and provide a consistent staining pattern in control lymphoid tissues. The specificity of immunostaining for Bcl-2 is further confirmed by the following observations. First, the staining is blocked with the synthetic peptide against which the antibodies were raised, and second, antibodies of the same IgG1 class, such as vimentin (Bionuclear, UK), do not stain mitotic cells. It is unlikely that the antibodies recognize an epitope(s) shared with another molecule with a similar M_r since both mono- and polyclonal antibodies gave the same result and no additional bands are observed by immunoblotting. Nuclear Bcl-2 expression is not readily seen in vivo perhaps because in these physiological circumstances it is present at a low level and is a more localized phenomenon that is thus consequently difficult to demonstrate by immunocytochemistry. The expression may be up-regulated by some yet unknown agents in vitro. Other factors may also contribute to the observed differences in mitosis-associated Bcl-2 expression. In the epithelial cell lines, immunostaining intensity increases rapidly when cells enter mitosis and decreases sharply after anaphase. This suggests that Bcl-2 might be associated with a chromatin-related molecule(s), resulting in changes of the epitope or accessibility to the antibodies and thus the immunoreactivity.

In summary, we have demonstrated that Bcl-2 protein is located in mitotic nuclei of epithelial cell lines. We speculate that this pattern of expression may indicate a special role in the protection of mitotic cells from programmed cell death and thus be involved in cell survival and immortalization in vitro.

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