ANTICANCER RESEARCH *24*: 2503-2510 (2004)

Oncogenes Modulate Cell Sensitivity to Apoptosis Induced by Glucose Deprivation

MAYA KANSARA and MICHAEL V. BERRIDGE

Malaghan Institute of Medical Research, PO Box 7060, Wellington South, New Zealand

Abstract. *Background: Increased glucose uptake is characteristic of many tumours and transformed cells and, consequently, glucose deprivation would be expected to differentially affect cancer cell survival and growth. In this study we investigated whether specific oncogenes sensitise cells to apoptosis induced by glucose deprivation. Materials and Methods: Oncogene-transformed 32D cells were deprived of glucose with or without IL-3 and [^{3}H]2-deoxyglucose uptake measured. Apoptosis was determined by AnnexinV/propidium iodide staining, cell cycle distribution was analysed and MYC expression determined by Western blotting. Results: v-Hras and to some extent v-src and v-abl enhanced apoptosis induced by glucose deprivation in the presence of IL-3 but attenuated apoptosis in its absence. In contrast, bcr-abl was highly protective against glucose deprivation-induced apoptosis for 72 hours in the presence and absence of IL-3, while bcl-2 was mildly protective in the presence of IL-3. With strongly transforming oncogenes, c-MYC expression correlated with cell sensitivity to apoptosis induced by glucose deprivation. Conclusion: Oncogenes vary markedly in their ability to protect cells from apoptosis following glucose deprivation and, in some situations, promote apoptosis.*

Cells must maintain a constant supply of energy to avoid apoptosis. Should intracellular ATP levels drop to 10-15% of those found in normal cells, a pre-existing program for apoptosis will be activated (1,2). However, maintenance of energy balance is not the sole regulatory point governing cell survival, since glucose, ATP and other metabolites cannot reverse apoptosis induced in various ways in haemopoietic cells. Survival of cells is a highly coordinated process whereby signal transduction pathways integrate both external and internal signals with metabolic pathways to ensure that energy levels meet cellular requirements. A common feature of growth factor/cytokine signalling is the ability to promote glucose uptake across the plasma membrane (3,4) and to stimulate cellular metabolism. Because cell transformation often overrides growth factor dependence, it is not surprising that cancer is often associated with increased glucose uptake. Increased glucose uptake has been attributed to increased glucose transporter expression (5), translocation (6) and intrinsic activation of glucose transporters (3,7,8). For example, the expression of *ras* (5), *abl* (9) or *fps* (10) has been found to stimulate glucose uptake in fibroblasts and haemopoietic cell lines.

Increased glucose uptake has been demonstrated in many different types of cancers, with advanced and aggressive tumours consuming greater quantities of glucose than early or benign tumours (1,11-13). The observation that transformed cells utilise aerobic glycolysis to metabolise glucose was made almost 80 years ago by Warburg (14). This form of metabolism produces only 2 molecules of ATP compared with 36 during oxidative phosphorylation and is usually associated with high rates of glucose uptake. Increased glucose uptake by cancer cells is commonly employed to locate and assess metabolic activity using [^{18}F]2-deoxyglucose and positron emission tomography. Transformed cells often maintain high levels of glucose uptake by over-expressing glucose transporter subtypes. For example, elevated levels of Glut-1 have been reported in a wide range of human malignancies including bladder, lung, ovary, testis, skin and breast (15-19), elevated Glut-2 in hepatic, pancreatic and breast carcinomas, and Glut-3 in high-grade gliomas (20). In addition, atypical expression of glucose transporter subtypes 4 and 5 has been observed in breast cancers (21).

The metabolic differences between cancer cells and normal cells remain attractive targets for anti-cancer

Abbreviations: IL-3, interleukin-3; Gluts, glucose transporters; 2DOG, 2-deoxyglucose; [^{3}H]2DOG, [^{3}H]2-deoxy-D-glucose.

Correspondence to: Dr M.V. Berridge, Malaghan Institute of Medical Research, PO Box 7060, Wellington South, New Zealand. Tel: 64-4-385 5096 x 825, Fax: 64-4-389 5095, e-mail: mberridge@malaghan.org.nz

Key Words: Glucose deprivation, oncogenes, *src, ras, abl, bcr-abl, bcl-2,* IL-3, apoptosis.

therapies, although to date this approach has not been fully exploited. A few studies have shown promising results, *e.g.* regional administration of the non-metabolisable glucose analog, 2-deoxyglucose, or "armed" glucose analogs, such as those conjugated to nitric oxide releasing agents, are novel approaches to target cancers that have high glucose consumption (22-24). In addition, suppression of Glut-1 mRNA expression using anti-sense has been demonstrated to suppress tumour growth (25). As well as targeted approaches, indirect evidence suggests that the overall reduction in glucose, *i.e.* caloric restriction, reduces tumour incidence and growth (26,27), while hypoglycaemic shock has been shown to preferentially kill cancer cells due to their increased dependence on glucose (28).

While previous studies have found a correlation between the introduction of an active oncogene and increased glucose uptake, the effects of specific oncogenes on cell sensitivity to apoptosis induced by glucose deprivation has not been determined. In this study we investigated whether transformation of 32D cells with *v-Hras, v-src, v-abl, bcr-abl* or *bcl-2* sensitises these cells to apoptosis induced by glucose deprivation and whether IL-3 alters this response. We show that these oncogenes differ widely in their ability to protect cells from glucose deprivation-induced apoptosis in the absence of IL-3 and that *v-Hras,* and to some extent *v-src* and *v-abl*, promote apoptosis in the presence of growth factor

Materials and Methods

Cells. 32Dcl3 and cl23 cells, originally derived by Greenberger (29), have been used widely to study transforming oncogenes. 32Dcl3 cells transformed with *v-src, v-abl* or *v-Hras* (JCS2) constructs were kindly provided by Dr S.M. Anderson (Department of Pathology, University of Colorado Health Science Centre, Colorado, USA). Cells were cultured at 37˚C in a humidified incubator maintained at 5% CO_2. Exponentially growing 32Dcl23 cells were transfected with the *bcr/abl* construct pSLXCMVbcr/abl (Dr S.M.Anderson), or with the *bcl-2* construct, PEF Bcl-2-2pGKpuropA (Dr A Strasser, Walter and Eliza Hall Institute, Melbourne, Australia) by electroporation using a Cell-Porator (GIBCO-BRL, Grand Island, NY, USA). For stable transfection, cells were cultured in RPMI 1640 containing 5% FBS for 48 h, and then selected by the addition of 1 mg/ml G418 (GIBCO-BRL) for 7 days. Cells were maintained as polyclonal populations to avoid introduction of clonal artefacts. All oncogene-transfected cells were maintained in RPMI 1640 supplemented with 10% FBS. *Bcl-2*-transfected cells were supplemented with 10% Wehi-3-conditioned medium to provide IL-3 as *bcl-2* did not confer growth factor independence. Morphologically, *v-ras-, v-src-*, or *v-abl*-transformed cells were similar to parental cells which were non-adherent and rounded in appearance under the phase contrast microscope. In contrast, *bcr/abl*-transformed cells were non-adherent and elongated with small dendrite-like processes *(results not shown)*.

Glucose deprivation. 32D cells were washed three times in glucose-free RPMI, then cultured in the same medium supplemented with 10% dialysed FBS and 1 mM sodium pyruvate. FBS (40 ml), was dialysed (Mr 10 kDa cut-off) at 4˚C against two 1L changes of PBS pH 7.4. Dialysed FBS was calculated to contain less than 1% glucose and was capable of supporting the long-term growth of 32D cells when supplemented with glucose and IL-3.

Glucose uptake. Glucose uptake was measured by the zero-trans method. Cells (10^6) were suspended in 250 µl glucose-free RPMI 1640 (GF-RPMI) without serum and preincubated for 3 min at 37˚C. [^{3}H] 2-deoxy-D-glucose (0.5 µCi) (Amersham, Little Chalfont, England) and 2-DOG were added at a final concentration of 100 µM. Uptake was determined at 37˚C for 3 min under conditions where glucose uptake was linear. Uptake was terminated by adding 250 µl ice-cold GF-RPMI containing 0.6 mM phloretin (Sigma-Aldrich, Castle Hill, Australia) and centrifuging through a cushion of 10% (w/v) BSA (50µl) at 8880 x g for 1 min in a microcentrifuge tube. The cell pellet was washed with 250 µl GF-RPMI, lysed in 100 µl of 0.1% Triton X-100, and radioactivity determined using a liquid scintillation spectrometer. Results are presented as the average of duplicate determinations ± S.E.M.

Cellular DNA content analysis. Cells (1 x 10^6) were washed, fixed with 70% ice-cold ethanol and stored overnight at 4˚C. Cells were pelleted and suspended in 1 ml 50 µg/ml propidium iodide (Sigma-Aldrich), 100 Kunitz units RNase A/ml (Sigma) in Mg^{2+} and Ca^{2+}-free PBS pH 7.4 containing 1 mg/ml glucose and left at room temperature for at least 30 min. Samples were analysed within 24 h in a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Cell cycle analysis was carried out using the ModFit LT version 1.0 cell cycle analysis software (Verity Software House, Topsham, ME, USA).

Measurement of apoptosis. Apoptosis was measured by flow cytometry using Annexin-V-FLUOS (Boehringer Mannheim, Germany). Cell staining was carried out according to the manufacturer's instructions, with minor modifications as follows: 10^6 cells were washed with PBS and centrifuged at 200 g for 5 min. Cells were incubated at room temperature for 10-15 min in 100 µl of buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 5 mM $CaCl_2$) containing 1 µl Annexin-V-FLUOS labelling solution and 2 µl 50 µg/ml PI). Cells were analysed using FACScan flow cytometer (Becton Dickinson) with FL1-H for Annexin-FLUOS and FL2-H for PI (30-32); sub-G1 cells were excluded from all analysis.

Detection of c-MYC by Western blotting. Cells were lysed in RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EGTA, 1 mM PMSF, 30 KIU/ml Aprotinin) (10^8 cells /ml) and incubated at 4˚C for 20 min. Lysates were clarified by centrifuging at 13,000 x g for 15 min in a Biofuge Fresco fixed rotor (Hereus, Hanan, Germany), mixed with Laemmli sample buffer and heated to 100˚C for 3 min. Proteins were separated using 10% SDS-polyacrylamide gels and transferred electrophoretically to supported nitrocellulose membranes (Hybond-C, Amersham) at 150 V for 45 min. Membranes were blocked with Tris-buffered saline (TBS) (150 mM NaCl, 50 mM Tris base, pH 7.4) containing 0.1% Tween 20 and 5% BSA for 1 h at room temperature and then incubated with 5 µg/ml anti-c-MYC (c-33) mAb IgG (Santa Cruz, CA, USA) for 1 h. The membranes were washed using TBS-Tween 20 and incubated with rabbit anti-mouse Ig conjugated to horseradish peroxidase (1:4000; DAKO, Glostrup, Denmark). Membranes were again washed in TBS-Tween and proteins detected using enhanced chemiluminescence

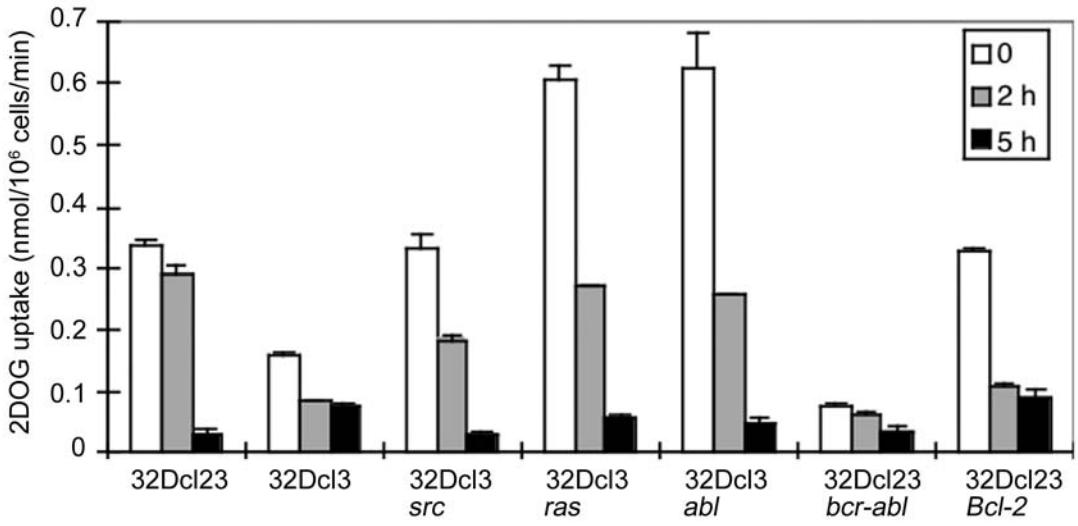

Figure 1. *Short-term effects of glucose deprivation on 2DOG uptake by normal and oncogene-transformed cells. [^{3}H] 2DOG (1 μCi, 100 μM) uptake was measured in exponentially growing cells and in cells subjected to glucose deprivation for 2 h or 5 h. Results are from one experiment and represent the mean of triplicate determinations ± S.E.M. The experiment shown is representative of two separate experiments.*

detection reagent (ECL, Amersham). Membranes were exposed to Hyperfilm-ECL™ for 30 sec to 5 min and the film developed using a Kodak RP-OMAT processor.

Results

To investigate the effects of specific oncogenes on the apoptosis induced by glucose deprivation, oncogene-transformed cells on a common haemopoietic cell background were employed. Murine 32Dcl3 cells stably transformed with *v-Hras, v-src* or *v-abl* had doubling times of 16 h, 15 h and 25 h compared with 17 h for 32Dcl3, while 32Dcl23 cells transformed with *bcr-abl* and transfected with *bcl-2* had doubling times of 26 h and 24 h compared with 25 h for 32Dcl23. Thus, except for *v-abl* which slowed cell cycling of 32Dcl3 cells, transformed cells showed comparable cell doubling times to parental cells.

Effect of glucose deprivation on 2DOG uptake by growth factor-dependent and oncogene-transformed 32D cell lines. Glucose uptake was measured in parental and oncogene-transformed 32D cells and the effects of glucose deprivation on 2DOG uptake determined (Figure 1). As expected, *src*-, *ras*- and *abl*-transformed 32Dcl3 cells exhibited 2-3.9 -fold greater 2DOG uptake than parental cells. Surprisingly, exponentially growing 32Dcl23*bcr/abl* cells exhibited only 23% of the 2DOG uptake observed with parental 32Dcl23 cells, whereas 32Dcl23*bcl-2* cells did not significantly differ from wild-type cells. Comparison of 2DOG uptake with cell doubling times showed no direct correlation, with 32Dcl23*bcr/abl* showing unexpectedly low and 32Dcl3*ras* unusually high 2DOG uptake. Thus, glucose uptake by oncogene-transformed 32D cells does not correlate with the predicted energy requirements for cell cycling.

The effect of glucose deprivation on glucose uptake by growth factor-dependent and oncogene-transformed 32D cell lines is also shown in Figure 1. 2DOG uptake declined by 20-63% following 2-h glucose deprivation and declined further after 5 h (50-91% of control). Reduced glucose uptake was not as pronounced with 32Dcl23 and 32Dcl23*bcr/abl* cells at 2 h, while uptake by 32Dcl3 and 32Dcl23*bcl-2* did not further decline at 5 h. In general, oncogenes did not maintain glucose uptake levels at those of glucose-maintained cells.

Oncogene sensitisation to apoptosis following glucose deprivation. The effects of specific oncogenes on glucose deprivation-induced apoptosis was investigated for periods of up to 72 h using Annexin V/propidium iodide staining and flow cytometry. Prior to glucose deprivation, cell viability was 90-98%. When 32Dcl3 and 32D cl23 cells were deprived of glucose in the absence of IL-3, cells rapidly became apoptotic with 20-25% survival at 24 h and 5-10% at 48 h (Figure 2). In the presence of IL-3, 56-60% of cell remained viable at 24 h and 25-40% at 48 h. Thus, IL-3 had a sparing effect on the apoptosis induced by glucose deprivation.

In the presence of specific oncogenes, glucose deprivation-induced apoptosis varied depending on the oncogene and whether growth factor was present. In the presence of IL-3, 32D cells transformed with *ras*, and to a much lesser extent *src* and *abl*, were more sensitive to apoptosis induced by glucose deprivation than were parental cells. In contrast, in

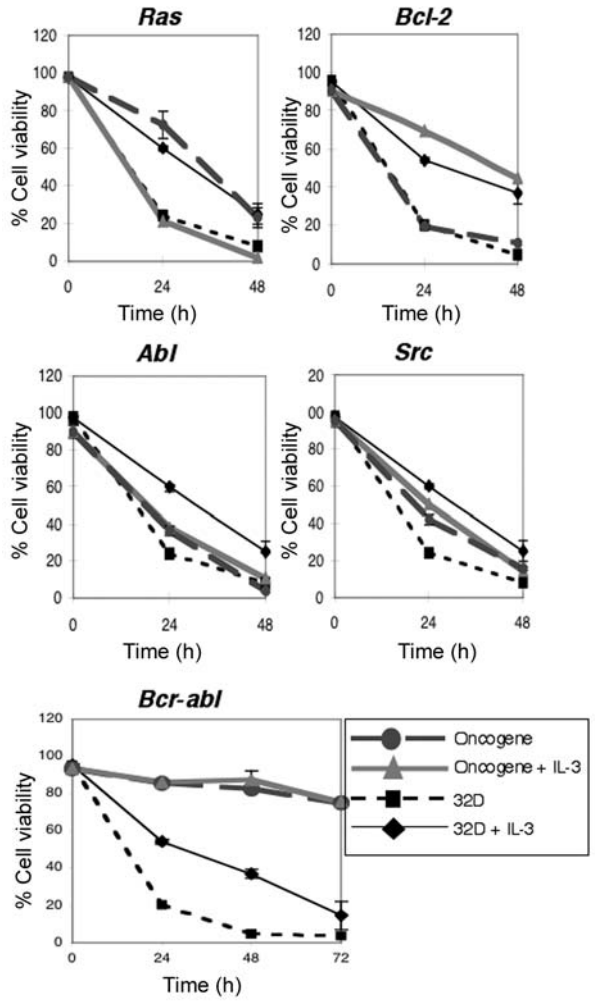

Figure 2. *Effects of oncogene expression on 32D cell survival in the presence or absence of IL-3 following glucose deprivation. Normal and oncogene-transformed 32D cells were cultured in GF-RPMI 1640 supplemented with 5% dialysed FBS ± 20 ng/ml IL-3 for up to 72 h. Cells were stained with Annexin V-FLUOS and PI and apoptosis determined by flow cytometry. 10,000 events were acquired using FLH-3 for PI and FLH-1 for Annexin-V-FITC. Cells negative for both PI and Annexin V comprised the viable cell population as plotted above. Results are the mean ± S.E.M from 3 separate experiments.*

the absence of IL-3, these oncogenes spared cells from glucose deprivation-induced apoptosis. With *bcl-2*, a small sparing effect on glucose deprivation-induced apoptosis was observed in the presence of IL-3 but no significant effect was seen in the absence of IL-3. In contrast to other oncogenes, *bcr-abl*-transformed 32D cells were remarkably resistant to glucose deprivation-induced apoptosis in the presence or absence of IL-3 maintaining 95% viability at 48 h and 85% viability at 72 h. This may be related to their low glucose uptake (22% that of parental cells (Figure 1), suggesting that these cells may utilize alternative energy sources.

Cell cycle changes following glucose deprivation in non-transformed and transformed cell lines. To investigate whether cell survival following glucose deprivation was associated with cell cycle arrest, cells were deprived of glucose for up to 72 h and cell cycle profiles determined by propidium iodide staining and flow cytometry. Cell cycle analysis was carried out on all viable cells and the percentage of viable cells is shown at the base of each histogram (Figure 3).

Following glucose deprivation, 32Dcl3 and 32Dcl3*src* arrested in G1, whereas 32Dcl23 arrested in both G1 and G2/M. 32Dcl3*ras*, 32Dcl3*abl*, 32Dcl23*bcl-2* and 32Dcl23*bcr/abl* failed to consistently accumulate in G1, with all cells except 32Dcl3*ras* showing a small tendency to arrest in G2 /M. Thus, in most cell lines examined, G1 and/or G2/M checkpoints appeared to be partially or fully intact. 32Dcl23*bcl-2* and 32Dcl3*abl* did not show a significantly altered proportion of cells in S-phase following glucose deprivation, whereas 32Dcl3ras showed a small increase and 32Dcl23*bcr/abl* a small decrease. Loss of cells from one phase of the cell cycle is normally balanced by changes in other cell cycle compartments. With 32Dcl23, 32Dcl3 and 32Dcl3*src* cells, loss of cells in S-phase was associated with increased G1 and/or G2/M and apoptosis. However, 32Dcl3*abl* cells that do not appear to lose cells from S-phase also undergo apoptosis.

c-MYC expression in normal and oncogene-transformed 32D cells. C-MYC participates in the control of cell growth, proliferation, programmed cell death and differentiation (33). The expression of c-MYC has been shown to sensitise cells to apoptosis following glucose deprivation. Therefore, we investigated whether c-MYC expression correlated with glucose deprivation–induced apoptosis in growth factor-dependent and oncogene-transformed cells. Cells were lysed and proteins separated on SDS-PAGE gels, transferred to nitrocellulose and probed with anti-c-MYC antibody. Under normal culture conditions, 32Dcl23, 32Dcl3, 32Dcl3*src* and 32Dcl3*abl* expressed high levels of c-MYC, whereas 32Dcl3*ras*, 32Dcl23*bcl-2* and 32Dcl23*bcr/abl* cells expressed considerably lower levels. Following 2 h of glucose deprivation, c-MYC expression decreased in all cell lines (Figure 4) and was barely detectable in 32Dcl23, 32Dcl3*ras*, 32Dcl3*abl*, 32Dcl23*bcr/abl* and 32Dcl23*bcl-2*. Interestingly, when compared with cell viability data (Figure 2), the low c-MYC-expressing cells, 32Dcl3*ras* and 32Dcl23*bcr/abl* were less sensitive to glucose deprivation-induced apoptosis than the high c-MYC-expressing cells (32Dcl3*src* and 32Dcl3*abl*).

Discussion

Many different cancer cells exhibit an increased rate of glucose uptake suggesting that this trait confers a survival benefit or proliferative advantage. Previous studies have demonstrated that glucose deprivation can cause cancer cells to apoptose

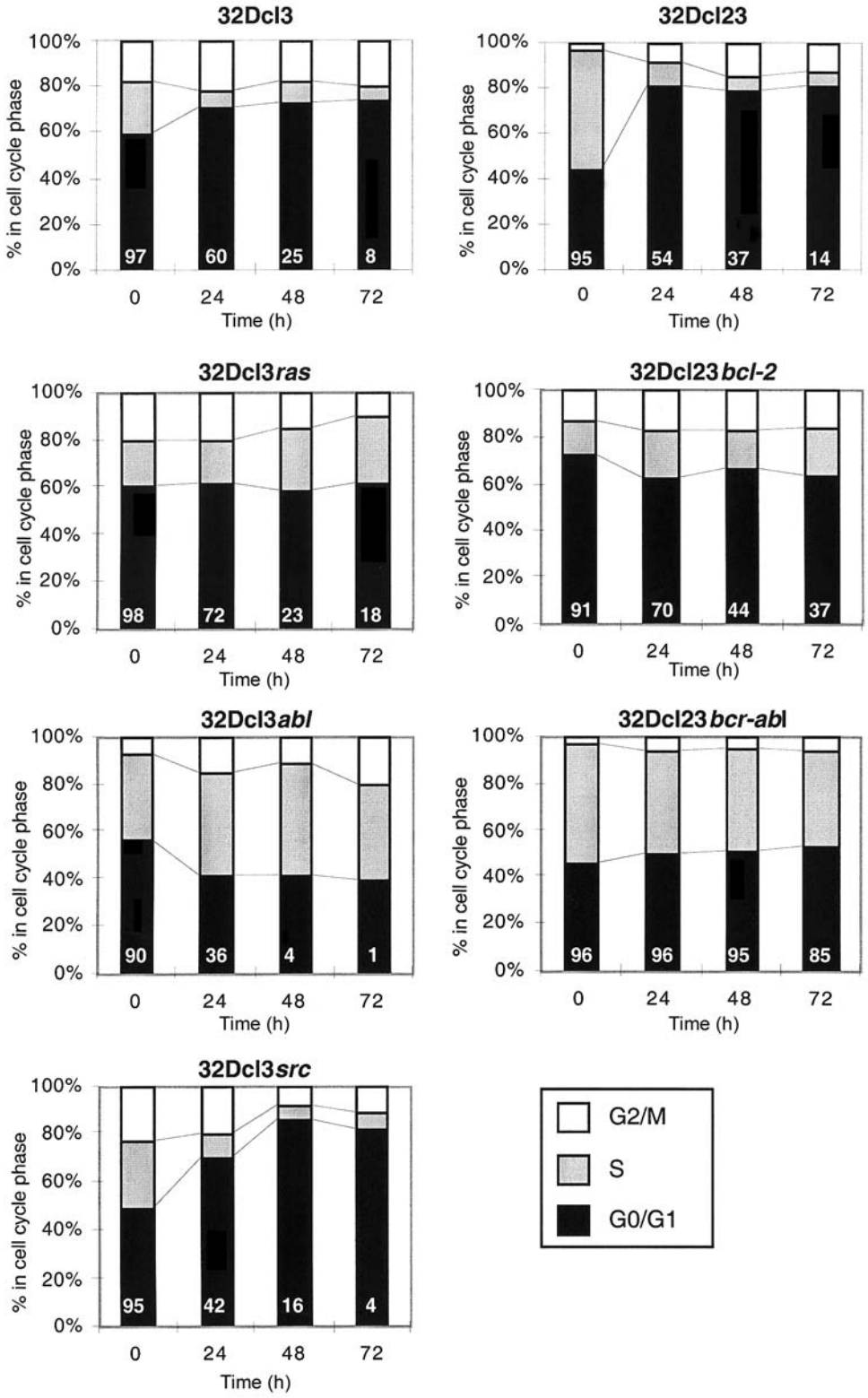

Figure 3. *Effect of glucose deprivation on cell cycle phase distribution in normal and transformed 32D cells. Cells were cultured in GF-RPMI 1640, 5% dialysed FBS and supplemented with 20 ng/ml IL-3 if required for normal cell growth (32Dcl23, 32Dcl3, 32Dcl23bcl-2). Cell samples were removed at the times indicated and cell distribution and DNA content determined by PI staining and flow cytometry and ModFit LT analysis. The percentage of viable cells is indicated at the bottom of each bar. The results of one experiment are shown and are representative of 3 separate experiments.*

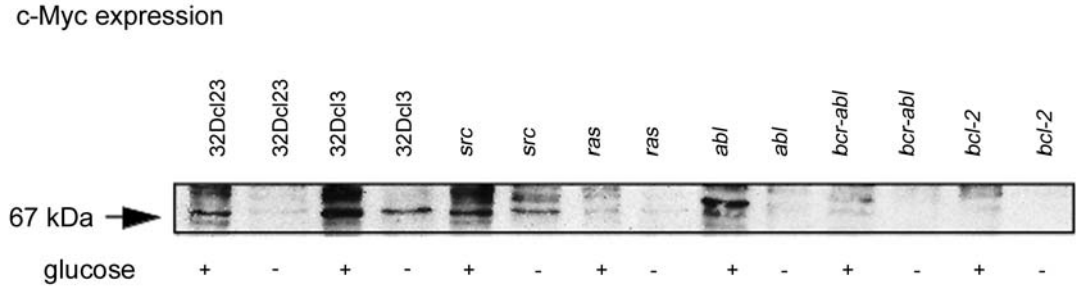

Figure 4. *c-MYC expression in normal and oncogene-transfected 32D cells. Cells were lysed and proteins resolved on SDS-PAGE gels, transferred to nitrocellulose and probed using anti-c-MYC antibody.*

(7,34,35). In this study we investigated whether transformation of 32D cells with specific oncogenes sensitised or protected cells from apoptosis induced by glucose deprivation and found that the effect observed depended on the particular oncogene.

Previous studies have shown that transfection of 32Dcl3 cells with constitutively activated *v-Hras, v-src* (36) *bcr/abl* (37), or *v-abl* (38) overcomes the need for IL-3 for survival and proliferation. *Bcl-2* expression on the other hand did not confer growth factor independence on 32D cells (39). Transformation of 32D cells with *ras*, which encodes a GTP-binding protein, or *src* or *abl*, which encode tyrosine kinases, increased their rate of glucose uptake compared with parental cells. In contrast, 32D cells expressing *bcl-2* did not alter glucose uptake, while 32D cells expressing *bcr-abl* exhibited decreased glucose uptake (22% of parental cells).

When parental 32D cells were deprived of glucose they underwent apoptosis and this was accelerated in the absence of IL-3. In contrast, 32D cells transformed with *ras* and to a much lesser extent *src* and *abl* in the presence of IL-3 were more sensitive to glucose deprivation-induced apoptosis than parental cells. In the absence of growth factor, *ras, src* and *abl* protected cells from glucose deprivation-induced apoptosis. Ras transformed cells were highly sensitised to glucose deprivation-induced apoptosis if IL-3 was present in the media. A similar phenomenon has been observed with MDA-468, human breast cancer cells, where treatment with EGF at low glucose concentrations appeared to sensitise these cells to undergo apoptosis (40). Interestingly, if 32Dcl3*ras* cells were cultured in the presence of both IL-3 and glucose, differentiation into macrophage-like cells was induced *(results not shown)*.

Bcl-2-transformed cells showed a similar loss of cell viability to parental cells when deprived of glucose and IL-3, but in the presence of IL-3, cell survival was enhanced. Previously *bcl-2* has been shown to block apoptosis at a point downstream from the collapse of cellular homeostasis (41). In neurons and astrocytes, overexpression of *bcl-2* and *bcl-xL* was shown to protect these cells from glucose deprivation-induced apoptosis (42). Thus, the protection offered by some *bcl-2* family members appears to be cell-type specific and may confer greater protection to primary cells rather than cell lines.

Interestingly, *bcr/abl*-transformed 32D cells maintained 95% viability at 48 h and 85% following 72 h of glucose deprivation, regardless of whether IL-3 was present in the culture media or not. The rate of glucose uptake in these cells was only 22% that of parental cells, suggesting that these cells require little glucose and are able to employ alternative energy sources. Recent studies have indicated that rapidly proliferating cancer cells require a constant supply of glucose whereas slower growing cells may not be so dependent on glucose (43). However, the cell division rates for both *bcr/abl* and parental cells were similar, indicating that glucose uptake is not the primary determinant of cell proliferation rate in these cells. Several reports have shown that, in the absence of glucose, ATP can be generated from pyruvate oxidation or from the conversion of glutamine to lactate (44,45). In a preliminary study, culturing 32Dcl23*bcr/abl* cells in the absence of both glutamine and glucose for 48 h did not result in cell death, nor did exposure to 11 mM 2DOG for 48 h (results not shown). The possibility that 32Dcl23*bcr/abl* cells generate ATP by mitochondrial metabolism of amino acids remains to be investigated (46,47).

Growth factor-dependent and transformed cells deprived of glucose were unable to maintain 2DOG uptake. None of the cells examined maintained 2DOG uptake at levels observed prior to glucose deprivation. Several different primary cell types have been found to up-regulate 2DOG uptake 2-to 4-fold following glucose deprivation (48-52), by increasing glucose transporter mRNA and cell surface protein expression. Cancer cells on the other hand respond to glucose deprivation initially by expressing an array of stress-related genes and subsequently undergoing apoptosis (53). For example, early in glucose deprivation, human breast cancer cell lines exhibit increased levels of Lyn and JNK as well as increased expression of c-MYC mRNAs. This suggests that some cancers cells are uniquely sensitive to glucose deprivation-induced apoptosis. Interestingly, except for 32Dcl23*bcl-2*, which requires IL-3 for survival and growth, those cells most sensitive to glucose deprivation-induced apoptosis were found to have high c-MYC expression. This has now been shown for several cell types, and apoptosis appears to be p53-independent and possibly linked to increased LDH-A expression (34).

Following glucose deprivation both parental and transformed cells accumulated in G1 or G2/M. Thus, in all cell lines examined, G1 and/or G2/M checkpoints were partially or fully intact. Existence of an energy-dependent checkpoint at both the G1/S and G2/M boundary has been reported before (2). These checkpoints, in theory, would give cells the ability to detect nutritional availability throughout the cell cycle.

As discussed, one major biochemical difference between normal and cancer cells is the way they take up and utilise glucose and therapeutic strategies for targeting high glucose-consuming cells are being developed (24). Our work clearly shows that cells expressing different oncogenes, cultured in the absence and presence of growth factors, respond differently when deprived of glucose. Cells expressing constitutively active *v-ras*, and to some extent *v-src* and *v-abl,* were more susceptible to cell death following glucose deprivation in the presence of IL-3 than parental cells, suggesting that cancers with these oncogenes may respond to treatment with compounds which block glucose uptake or its metabolism *i.e.* 2DOG, while cancers expressing *bcr/abl* or *bcl-2* may not be susceptible to these therapies.

It is important to note that the transforming events investigated in this study are on an immortalised cell background and that combined effects of oncogenes and/or tumour suppressors will be operative. The immortalisation events in 32Dcl23 and 32Dcl3 are unknown; it would be interesting to determine whether cancer cells known to carry specific oncogenes behave like oncogene-transfected 32D cells when deprived of glucose. Our study suggests that therapies which take advantage of increased glucose consumption (*e.g.*, inhibition of glucose transport or non-metabolisable glucose analogues) should take into account the particular transforming event. Although full tumour genotypes are seldom known, some tumours are characterised by overexpression of specific oncogenes. For instance, *bcr/abl* is found in 90-95% of CML (54), and mutations of the *ras* proto-oncogenes (*H-ras, N-ras* and *K-ras*) are found in 20–30% of all human tumours including 90% of adenocarcinomas of the pancreas, and 50-70% of CML. Together, these data suggest that detailed knowledge of the cancer genotype and phenotype may benefit patients being treated with inhibitors of glucose transport, and that some tumours may be more or less responsive than others. Thus, under conditions where growth factor is limiting, cancers containing *bcr/abl, ras* and *bcl-2* may be more resistant to therapies where glucose transport is inhibited. Ideally, an inhibitor of glucose uptake or metabolism that is toxic in high doses but does not affect normal tissues and does not pass through the blood brain barrier is desirable. Treatment of certain cancers with inhibitors of glucose uptake in conjunction with localised treatment with current therapies may prove to be highly beneficial in some situations.

Acknowledgements

This work was supported by the Cancer Society of New Zealand and its Wellington Division and by the Health Research Council of New Zealand.

References

1 Nieminen AL, Saylor AK, Herman B and Lemasters JL: ATP depletion rather than mitochondrial depolarization mediates hepatoctye killing after metabolic inhibition. Am J Physiol *267*: C67-C74, 1994.
2 Sweet S and Singh G: Accumulation of human promyelocytic leukemic (HL-60) cells at two energetic cell cycle checkpoints. Cancer Res *55*: 5164-7, 1995.
3 Berridge MV and Tan AS: Interleukin-3 facilitates glucose transport in a myeloid cell line by regulating the affinity of the glucose transporter for glucose: involvement of protein phosphorylation in transporter activation. Biochem J *305*: 843-851, 1995.
4 McCoy KD, Ahmed N, Tan AS and Berridge MV: The hemopoietic growth factor, interleukin-3, promotes glucose transport by increasing the specific activity and maintaining the affinity for glucose of plasma membrane glucose transporters. J Biol Chem *272*: 17276-17282, 1997.
5 Flier JS, Mueckler MM, Usher P and Lodish HF: Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. Science *235*: 1492-1495, 1987.
6 Young AT *et al*: Phosphatidylinositol 3-kinase binding to polyoma virus middle tumor antigen mediates elevation of glucose transport by increasing translocation of the GLUT1 transporter. Proc Natl Acad Sci USA *92*: 11613-7, 1995.
7 Kan O, Baldwin SA and Whetton AD: Apoptosis is regulated by the rate of glucose transport in an interleukin 3 dependent cell line. J Exp Med *180*: 917-23, 1994.
8 Tan AS, Ahmed N and Berridge MV: Acute regulation of glucose transport after activation of human peripheral blood neutrophils by phorbol myristate acetate, fMLP, and granulocyte-macrophage colony-stimulating factor. Blood *91*: 649-55, 1998.
9 Ahmed N and Berridge MV: Transforming oncogenes regulate glucose transport by increasing transporter affinity for glucose: contrasting effects of oncogenes and heat stress in a murine marrow-derived cell line. Life Sci *63*: 1887-903, 1998.
10 Birnbaum MJ, Haspel HC and Rosen OM: Transformation of rat fibroblasts by FSV rapidly increases glucose transporter gene transcription. Science *235*: 1495-1498, 1987.
11 Di Chiro G *et al*: Glucose utilization of cerebral gliomas measured by (18F) fluorodeoxyglucose and positron emmison tomography. Neurology *32*: 1323-1329, 1982.
12 Kato H and Takita J: Glut-1 glucose transporter expression in esophagela squamous cell carcinoma is associated with tumor aggressiveness. Anticancer Res *22*: 2635-2640, 2002.
13 Nolop K *et al*: Glucose utilization *in vivo* by human pulmonary neoplasms. Cancer *60*: 2682-2689: 1987.
14 Warburg O: On the origins of cancer cells. Science *123*: 309-314, 1956.
15 Grover-McKay M, Walsh SA, Seftor EA, Thomas PA and Hendrix MJ: Role for glucose transporter 1 protein in human breast cancer. Path Onc Res *4*: 115-20, 1998.
16 Haber RS, Weiser KR, Pritsker A, Reder I and Burstein DE: GLUT1 glucose transporter expression in benign and malignant thyroid nodules. Thyroid *7*: 363-367, 1997.

17 Ito T *et al*: Expression of facilitative glucose transporter isoforms in lung carcinomas: its relation to histologic type, differentiation grade, and tumor stage. Modern Pathol *11*: 437-433, 1998.
18 Younes M, Brown RW, Mody DR, Fernandez L and Laucirica R: GLUT1 expression in human breast carcinoma: correlation with known prognostic markers. Anticancer Res *15*: 2895-8, 1995.
19 Younes M, Lechago LV, Somoano JR, Mosharaf M and Lechago J: Wide expression of the human erythrocyte glucose transporter Glut1 in human cancers. Cancer Res *56*: 1164-7, 1996.
20 Boado RJ, Black KL and Pardridge WM: Gene expression of GLUT3 and GLUT1 glucose transporters in human brain tumors. Brain Research. Molecular Brain Res *27*: 51-7, 1994.
21 Binder C, Binder L, Marx D, Schauer A and Hiddemann W: Deregulated simultaneous expression of multiple glucose transporter isoforms in malignant cells and tissues. Anticancer Res *17*: 4299-4304, 1997.
22 Cay O, Radnell M, Jeppsson B, Ahren B and Bengmark S: Inhibitory effect of 2-deoxy-D-glucose on liver tumor growth in rats. Cancer Res *52*: 5794-5796, 1992.
23 Colquhoun A *et al*: Novel monosaccharides as potent inhibitors of cell proliferation. Cell Biochem Funct *15*: 243-9, 1997.
24 Cantuaria G *et al*: Antitumor activity of a novel glyco-nitric oxide conjugate in ovarian carcinoma. Cancer *88*: 381-8, 2000.
25 Noguchi Y *et al*: Suppression of facilitative glucose transporter 1 mRNA can suppress tumor growth. Cancer Lett *154*: 175-82, 2000.
26 Kritchevsky D: Caloric restriction and experimental carcinogenesis. Toxicol Sci *52*: 13-6, 1999.
27 Yoshida K, Inoue T, Nojima K, Hirabayashi Y and Sado T: Calorie restriction reduces the incidence of myeloid leukemia induced by a single whole-body radiation in C3H/He mice. Proc Natl Acad Sci USA *94*: 2615-2619, 1997.
28 McCarty MF: The therapeutic potential of glucose tolerance factor. Med Hypotheses *6*: 1177-89, 1980.
29 Greenberger JS, Sakakeeny MA, Humphries RK and Lunenfeld B: Demonstration of permanent factor-dependent multipotential (erythroid/neutrophil/basophil) hematopoietic cell lines. Proc Natl Acad Sci USA *80*: 2931-2935, 1983.
30 Van Engeland M, Ramaekers FC, Schutte B and Reutelingsperger CP: A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherent cells in culture. Cytometry *24*: 131-9, 1996.
31 Van Engeland M, Nieland LJ, Ramaekers FC, Schutte B and Reutelingsperger CP: Annexin V-affinity assay: a review on an apoptosis detection system based on phosphatidylserine exposure. Cytometry *31*: 1-9, 1998.
32 Vermes I, Haanen C, Steffens-Nakken H and Reutelingsperger C: A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods *184*: 39-51, 1995.
33 Amati B *et al*: Transcriptional activation by the human c-Myc oncoprotein in yeast requires interaction with Max. Nature *359*: 423-426, 1992.
34 Shim H, Chun YS, Lewis BC and Dang CV: A unique glucose-dependent apoptotic pathway induced by c-Myc. Proc Natl Acad Sci USA *95*: 1511-6, 1998.
35 Lee Y, Galoforo S, Berns C, Tong W, Kim H and Corry P: Glucose deprivation-induced cytotoxicity in drug resistant human breast carcinoma MCF7/ADR cells. J Biol Chem *272*: 11690-11693, 1997.
36 Kruger A and Anderson SM: The *v-src* oncogene blocks the differentiation of a murine myeloid progenitor cell line and induces a tumorigenic phenotype. Oncogene *6*: 245-256, 1991.
37 Laneuville P, Timm M and Hudson AT: bcr/abl expression in 32D cl3(G) cells inhibits apoptosis induced by protein tyrosine kinase inhibitors. Cancer Res *54*: 1360-6, 1994.
38 Rovera G, Valtieri M, Mavilio F and Reddy EP: Effect of Abelson murine leukemia virus on granulocytic differentiation and interleukin-3 dependence of a murine progenitor cell line. Oncogene *1*: 29-35, 1987.
39 Nunez G *et al*: Deregulated Bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. J Immunol *144*: 3602-10, 1990.
40 Kaplan O *et al*: Toxicity and the effects of epidermal growth factor on glucose metabolism of MDA-468 human breast cancer cells. J Biol Chem *265*: 13641-13649, 1990.
41 Maher F and Harrison LC: Hexose specificity for downregulation of HepG2/brain-type glucose transporter gene expression in L6 myocytes. Diabetologia *33*: 641-8, 1990.
42 Xu L, Koumenis IL, Tilly JL and Giffard RG: Overexpression of bcl-xL protects astrocytes from glucose deprivation and is associated with higher glutathione, ferritin, and iron levels. Anesthesiology *91*: 1036-46, 1999.
43 Singh G, Lakkis CL, Laucirica R and Epner DE: Regulation of prostate cancer cell division by glucose. J Cell Physiol *180*: 431-8, 1999.
44 Zielke HR, Ozand PT, Tildon JT, Sevdalin DA and Cornblath M: Reciprocal regulation of glucose and glutamine utilization by cultured human diploid fibroblasts. J Cell Physiol *95*: 41-48, 1978.
45 Mazurek S, Boschek CB and Eigenbrodt E: The role of phosphometabolites in cell proliferation, energy metabolism, and tumor therapy. J Bioenerg Biomembr *29*: 315-330, 1997.
46 Pederson P: Tumour Mitochondria and the Bioenergetics of Cancer Cells. (ed. Homburger F) (Karger, Basel, 1978).
47 Kouvroukoglou S, Lakkis C, Wallace JD, Zygourakis K and Epner DE: Bioenergetics of rat prostate cell migration. Prostate *34*: 137-144, 1998.
48 Bruckner BA, Ammini CV, Otal MP, Raizada MK and Stacpoole PW: Regulation of brain glucose transporters by glucose and oxygen deprivation. Met: Clin Exptl *48*: 422-31, 1999.
49 Gaposchkin CG and Garcia-Diaz JF: Modulation of cultured brain, adrenal, and aortic endothelial cell glucose transport. Biochim Biophys Acta *1285*: 255-66, 1996.
50 Guillet-Deniau I, Leturque A and Girard J: Expression and cellular localization of glucose transporters (GLUT1, GLUT3, GLUT4) during differentiation of myogenic cells isolated from rat foetuses. J Cell Sci *107*: 487-96, 1994.
51 Haspel HC, Mynarcik DC, Ortiz PA, Honkanen RA and Rosenfeld MG: Glucose deprivation induces the selective accumulation of hexose transporter protein GLUT-1 in the plasma membrane of normal rat kidney cells. Mol Endocrinol *5*: 61-72, 1991.
52 Kosaki A *et al*: Regulation of glucose transporter synthesis in cultured human skin fibroblasts. Mol Cell Endocrinol *75*: 189-96, 1991.
53 Lee Y and Corry P: Metabolic oxidative stress-induced HSP79 gene expression is mediated through the SAPK pathway. J Biol Chem *273*: 29857-29863, 1998.
54 De Klein A *et al*: A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukemia. Nature *300*: 635-637, 1982.

Received February 25, 2004
Accepted May 10, 2004