Involvement of branched-chain amino acid aminotransferase (*Bcat1/Eca39*) in apoptosis

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Abstract The branched-chain amino acid aminotransferase, *Bcat1/Eca39*, catalyzes the first step of branched-chain amino acid catabolism. *Bcat1/Eca39* was originally isolated from a c-*myc*-induced tumor and was proven to be a direct target for c-Myc regulation. The gene is highly conserved in evolution and disruption of its yeast homolog affects cell growth. To assess the role of *Bcat1/Eca39* in mammalian cells, we overexpressed *Bcat1/Eca39* in murine cells and studied effects on cell growth. Overexpression of *Bcat1/Eca39* had no apparent effect on the proliferation of cells grown with high serum concentrations, but under serum deprivation conditions, led to a decrease in cell viability. Cell death under these conditions displayed apoptotic features. The branched-chain keto acid, α-ketoisocaproate, a metabolite of leucine catabolism produced by BCAT1/ECA39, was previously found to inhibit cell growth. We show that α-ketoisocaproate can induce rapid apoptotic cell death. This observation suggests that the growth inhibitory effect of BCAT1/ECA39 and its apoptosis promoting effect may be mediated by the levels of the products of BCAT1/ECA39 activity, namely, branched-chain keto acids.

Key words: Apoptosis; Branched-chain amino acid; Aminotransferase; Keto acid

1. Introduction

Catabolism of branched-chain amino acids (BCAA) is a major pathway in the metabolism of essential amino acids. This pathway allows the utilization of BCAA for energy purposes and as a source of various direct and indirect metabolites such as glutamate [1]. The significance of this pathway is best demonstrated in maple syrup urine disease (MSUD), where as a result of mutations in another enzyme in the pathway, BCAA and branched-chain keto acids (BCKA) accumulate, and the patients suffer from mental and physical retardation sometimes leading to coma and death [2]. Branched-chain amino acid aminotransferases (BCATs) are the enzymes regulating the first step in degradation of BCAA, and the genes coding for these enzymes were recently isolated [3,4]. *Bcat1/Eca39* codes for the cytosolic branched-chain amino acid aminotransferase and is expressed in a wide range of mammalian embryonic tissues [5–7], while in adult life it is mainly found in the brain and kidney [8,9]. The other BCAT isoenzyme (BCAT2/ECA40) is mitochondrial and is considered to be a housekeeping protein found in all tissues [5,8] and not sensitive to cell proliferation state [10].

The *Bcat1/Eca39* gene is directly regulated by c-Myc. It was isolated by a subtraction/co-expression strategy utilizing c-Myc-induced tumors from transgenic mice [7]. *Eca39* harbors a functional c-Myc binding motif and has been shown to be a direct target for c-Myc activation in both mouse and man [7,9,11]. c-*myc* is a major regulator of cell growth, differentiation and oncogenesis (for reviews see references [12–15]). Cell culture studies have shown that overexpression of c-*myc* can induce cells to enter the cell division cycle and proliferate [16,17]. Yet, overexpression of c-*myc* in serum deprived cultured cells can also induce apoptosis [18,19]. The *myc* family of proto-oncogenes codes for transcription factors [15,20,21] which together with another nuclear protein, Max, bind a specific DNA sequence and activate transcription [22,23]. The pathways by which c-Myc induces cellular processes such as proliferation, differentiation or apoptosis, are still obscure and only a small number of target genes, directly regulated by c-Myc, have been identified so far [15,24].

To study the function of *Eca39*, its possible effects on cell proliferation and its possible involvement in Myc-induced processes, we have recently characterized several homologs of *ECA39* from vertebrates and invertebrates [4,25,26]. We disrupted the *Saccharomyces cerevisiae* homolog of *Eca39* and found that yeast cells lacking *ECA39* grew faster due to shortening of the G1 stage of the cell division cycle [26]. These observations suggest that ECA39 may have an anti-proliferative effect on growth. In the present work, we study the involvement of *Bcat1/Eca39* in proliferation and apoptosis in mammalian cells.

2. Materials and methods

2.1. plasmids and cells

pPGK-ECA39, an expression vector in which *Bcat1/Eca39* cDNA is under the control of the phospho-glycerate kinase-1 (PGK-1) promoter was constructed by inserting a *Bbr*pI-*Xho*I 1292 bp fragment from the murine *Eca39* cDNA into the *Sma*I-*Xho*I sites of pCA1038 [27]. The PGK-neo plasmid [27] was used as a selective marker.

NIH3T3 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Biological Industries, Israel), 50 units/ml of penicillin G, 50 mg/ml of streptomycin and 4 mM L-glutamine. DNA was transfected into NIH3T3 cells by electroporation (960 μF, 250 V, 200 Ω, BioRad Gene Pulser) [28]. Transfectants were selected with 0.4 mg/ml G-418.

2.2. RNA isolation and Northern blot analysis

Total RNA was extracted using guanidine thiocyanate and centrifuged through a CsCl cushion by the method of Chirgwin et al. [29]. Northern blot analysis was performed as described [30]. DNA probes were labeled with a random priming kit (Boehringer Mannheim) using [γ-^{32}P]dCTP (3000 Ci/mM; ICN).

2.3. Expression of recombinant proteins and preparation of polyclonal antibodies against BCAT1/ECA39

Bcat1/Eca39 was expressed in bacteria using the QIAexpressionist

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system (Qiagen). A *Pst*I-*Xho*I 1.2 kb fragment of *Bcat1/Eca39* cDNA was cloned into the *Pst*I-*Hin*dIII sites of pQE32 producing an inducible expression vector coding for a His-tagged BCAT1/ECA39 protein. Expression was induced with 0.2 mg/ml IPTG for 3 h. The expressed protein appeared in inclusion bodies and was purified using His-Bind beads obtained from Novagen according to supplied protocol. The purified recombinant protein was used to produce polyclonal antibodies in rabbits according to standard procedures [31].

2.4. Cell extract preparation, BCAT activity assay and Western blot analysis

Cell extracts were prepared by homogenization as described [32]. Western blot analysis was performed as described by Towbin et al. [33]. Thirty μg protein from each extract were separated by SDS-PAGE in a 10% polyacrylamide gel. Polyclonal anti-BCAT1/ECA39 was used at 1/10 000 dilution as the primary antibody, and goat anti-rabbit IgG (whole molecule) alkaline phosphatase-conjugated (Sigma) was used as the secondary antibody. BCAT activity was assayed by measuring branched-chain keto acids produced using a colorimetric assay as previously described [4], except potassium phosphate pH 7.8 was used as buffer.

2.5. Proliferation rate, TUNEL and FACS analyses

Cell proliferation rate was determined using a WST-1 kit (Boehringer Mannheim), a colorimetric assay for mitochondrial dehydrogenases in viable cells. 3000 cells/well were plated in a 96-well plate with 10% fetal calf serum (FCS). Cells were allowed to grow for 16 h before cell viability was examined. Each point was measured in triplicate. For starvation, cells were initially plated and allowed to grow for 16 h in 10% FCS, before transferring them to fresh starvation medium with 0.2% FCS.

FACS analysis of cellular DNA content for estimation of cell cycle stage distribution, was performed as described [34]. Briefly, cells were fixed in ethanol, washed with PBS, treated with phosphate-citrate and Rnase A, and stained with propidium iodide.

TUNEL assay for detection of apoptotic cells using biotin-16-dUTP and ExtraAvidin-TRITC was performed as described [35]. Detection of TUNEL-stained nuclei by alkaline phosphatase was performed using a standard kit from Boehringer Mannheim.

3. Results

3.1. Overexpression of Bcat1/Eca39 in NIH3T3 cells

To obtain cell lines overexpressing *Bcat1/Eca39*, NIH3T3 cells were stably transfected with an expression vector containing the *Bcat1/Eca39* cDNA under the control of the PGK1 promoter. The PGK-neo vector was co-transfected to permit selection for G-418 resistant transfectants. Cells transfected with the PGK-neo vector alone were used as controls. Several resistant clones were isolated and expression of *PGK-Eca39* was confirmed in eight of them by Northern blot analysis with a *Bcat1/Eca39* specific probe (Fig. 1A). Since the expression vector lacks 0.9 kb of the 3′ untranslated region of the *Bcat1/Eca39* gene, the exogenous transcript appears smaller than the endogenous transcript (1.5 kb vs. 2.4 kb). Expression of the endogenous *Bcat1/Eca39* is dependent on the proliferative state of the cells; it is high in dividing cells and decreases in arrested cells (Fig. 1B and [10]). Expression of the exogenous *PGK-Eca39* gene does not seem to be dependent on cell proliferation. It is constitutively expressed in dividing cells and in cells arrested either by serum starvation or by allowing cells to grow to confluence (Fig. 1B).

Overexpression of BCAT1/ECA39 at the protein level was confirmed by Western blot analysis using an antibody developed against a recombinant BCAT1/ECA39 protein, expressed in bacteria (see Section 2). A band of about 40 kDa corresponding to the BCAT1/ECA39 protein was detected (Fig. 1C). An additional smaller faint band was also labeled, but this band was also evident when using the pre-immune serum, and thus could serve as a reference to compare the amounts of protein loaded on the gel (Fig. 1C).

Transamination of each of the three branched-chain amino

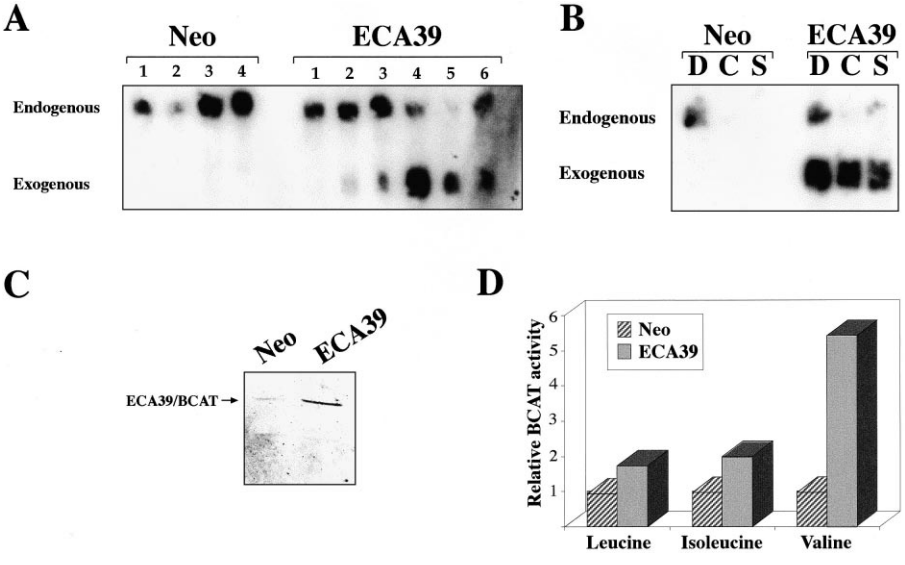

Fig. 1. Overexpression of *Bcat1/Eca39* in NIH3T3 cells. A: NIH3T3 cells were transfected with pPGK-ECA39 (ECA39) and PGK-neo, or only with the selection marker PGK-neo (Neo). Several stable clones were isolated from each experiment. Total RNA was prepared from the various clones, blotted and hybridized with a probe for *Bcat1/Eca39*. An endogenous transcript of 2.4 kb was recognized in all cells and a new transcript of about 1.5 kb was seen in most of the cell lines transfected with pPGK-ECA39. B: Expression of *Bcat1/Eca39* in different proliferation states: dividing cells (D), confluent cells (C) and serum-deprived cells (0.1% FCS) (S). Note that the endogenous gene is repressed in non-proliferative conditions, while the exogenous gene with the PGK1 promoter is not sensitive to the proliferative state. C: Western blot analysis of BCAT1/ECA39 expression. Thirty μg of protein from cell extracts from *Bcat1/Eca39* overexpressing cells (ECA39) and control cells (Neo) were separated by SDS-PAGE. Expression of *Bcat1/Eca39* was determined using an antibody raised against a recombinant BCAT1/ECA39 protein (see Section 2). D: BCAT activity in cells overexpressing *Bcat1/Eca39* (ECA39) and in control cells (Neo) grown for 24 h in 0.2% FCS. Activity was measured using leucine, isoleucine or valine as substrate. Results are given in units relative to the control activity for each substrate.

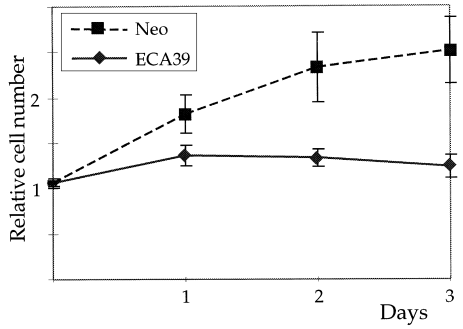

Fig. 2. Viability of cells overexpressing *Bcat1/Eca39* under serum starvation conditions. Growth rate of cells grown in DMEM with low serum (0.2% FCS) was followed for 3 days and estimated by WST-1 assay (described in Section 2). Each point represents measurements from four *Bcat1/Eca39* expressing cell lines (ECA39) or three control cell lines (Neo) done in triplicate (mean ± S.E.).

acids (leucine, valine and isoleucine) was examined in two clones. A 2–5-fold increase in BCAT activity was observed in extracts from cells transfected with the *Bcat1/Eca39* expression vector compared to control cells (Fig. 1D).

3.2. Proliferation rate of Bcat1/Eca39 overexpressing cells

To study the involvement of BCAT1/ECA39 in cell proliferation, we determined the growth rate of cells overexpressing *Bcat1/Eca39* in high or low serum conditions. In medium containing 10% FCS, no significant difference in growth rate between overexpressing and control cells was observed and no foci were detected when cells were allowed to grow to confluency (data not shown). However, in 0.2% FCS, the growth rate of *Bcat1/Eca39* overexpressing cells was significantly lower than that of the control cells (Fig. 2), indicating that *Bcat1/Eca39* overexpressing cells are more sensitive to serum starvation conditions. Some variations were observed between clones but average results from four different clones showed a significant ($P < 0.005$) decrease in cell viability of *Bcat1/Eca39* overexpressing cells and increased cell death after 2 days in low serum (Fig. 2).

3.3. Cell death in Bcat1/Eca39 overexpressing cells

Overexpression of c-*myc*, the regulator of *Bcat1/Eca39*, in fibroblasts induces apoptosis during serum starvation [18]. To determine whether the excessive cell death of serum-starved *Bcat1/Eca39* overexpressing cells was due to apoptosis we searched for apoptotic features in these cells compared to control cells. Cells were grown for 2 days in 0.2% FCS and cellular DNA content was determined by FACS analysis. The population of cells with sub-G1 DNA content, characteristic of apoptotic cells, was significantly larger in serum-starved *Bcat1/Eca39* overexpressing cells than that found in control cells (Fig. 3A). The fraction of sub-G1 cells varied between overexpressing clones (9–25%) with an average of 16 ± 4.6% (mean ± S.E.), and was significantly higher ($P < 0.05$) than in control cells (6 ± 0.3%).

To demonstrate DNA fragmentation in intact cells we performed a TUNEL assay in which terminal transferase is used to label free DNA ends. The assay detected excessive DNA fragmentation in nuclei of *Bcat1/Eca39* overexpressing cells (Fig. 3B). By counting cells in several fields we estimated that the nuclei of about 32% of *Bcat1/Eca39* expressing cells were positively stained by the TUNEL assay compared to only 3% of control cells (Fig. 3B). Since dying cells tend to disintegrate, some of them are lost during preparation of cells for FACS analysis. This, together with the better sensitivity of TUNEL compared to FACS, can explain the variation in the fraction of apoptotic cells identified by the two methods. In addition to DNA fragmentation, DAPI staining of serum-starved cells overexpressing *Bcat1/Eca39* demonstrated condensed chromatin in many nuclei (Fig. 3B), another frequent feature of apoptosis.

3.4. Effect of α-ketoisocaproate (KIC) on NIH3T3 cells

Eca39 codes for the metabolic enzyme branched-chain amino acid aminotransferase (BCAT, EC 2.6.1.42) [3,4]. This enzyme catalyzes the first step of branched-chain amino acid (BCAA) catabolism, transferring the amine group from the BCAA to α-ketoglutarate, producing glutamate and leaving the BCAA in their keto form.

The effect of *Bcat1/Eca39* overexpression on cell viability may be independent of its defined enzymatic activity. Alternatively, overexpression of BCAT may promote cell death through changes in the levels of metabolites involved in the

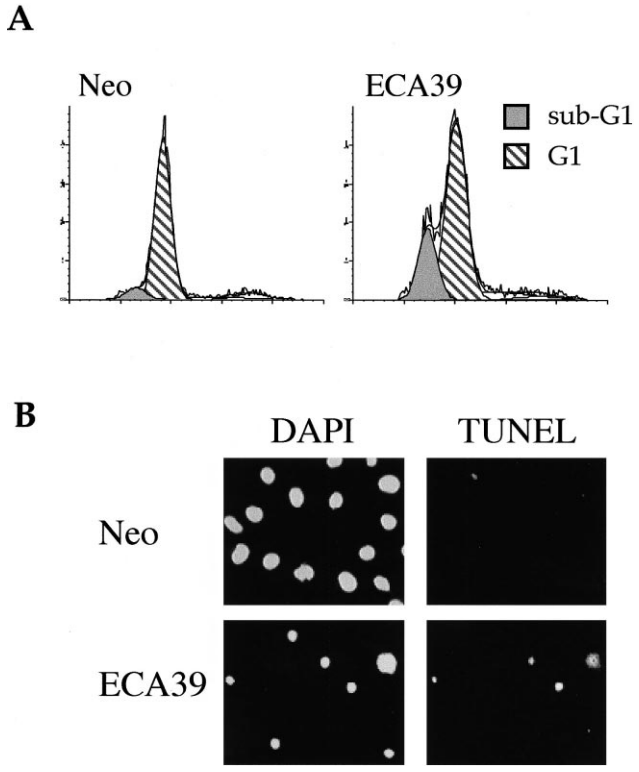

Fig. 3. Apoptotic features in serum-starved cells overexpressing *Bcat1/Eca39*. A: FACS analysis of *Bcat1/Eca39* overexpressing (ECA39) and control (Neo) cell lines after 2 days in DMEM with 0.2% FCS. The plots represent one clone of each type. To estimate the G1 population and apoptotic (sub-G1) population data from three clones of each type were analyzed using Modfit LT software. In *Bcat1/Eca39* overexpressing cells about 16 ± 4.6% (mean ± S.E.) of the cells appeared in the sub-G1 fraction compared to 6 ± 0.3% in control cells ($P < 0.05$). B: TUNEL assay for detection of apoptotic cells. *Bcat1/Eca39* expressing (ECA39) and control (Neo) cells were grown for 2 days in DMEM with 0.2% FCS, fixed and analyzed by TUNEL staining using biotin-16-dUTP and ExtraAvidin-TRITC. About 32% of nuclei were positively stained in *Bcat1/Eca39* expressing cells, compared to 3% in the control. DAPI staining was used to detect nuclei and observe their morphology.

transamination reaction. These metabolites include the branched-chain amino acids (leucine, isoleucine and valine), the branched-chain keto acids (α-ketoisocaproate, α-keto-β-methylvalerate and α-ketoisovalerate), glutamate and α-ketoglutarate. The effects of BCAA and BCKA on cells and tissues were analyzed in studies relating to the genetic disease known as maple syrup urine disease (MSUD), in which a defect in the second step of BCAA catabolism (dehydrogenation of BCKA) results in accumulation of BCAA and BCKA [2]. In these studies α-ketoisocaproate (KIC) was reported to inhibit cell proliferation and prolong the G1 stage of the cell division cycle [36]. We therefore studied the effects of BCAA and BCKA on proliferation of NIH3T3 cells and asked whether high levels of KIC can simulate *Bcat1/Eca39* overexpression and induce cell death even when cells are grown in high serum. As shown in Fig. 4, addition of KIC to the medium induced rapid cell death. Within 2–4 h from the addition of KIC, cells shrank, detached from the plate, assumed a round morphology and appeared more refractile.

Proliferation rates depended on the concentrations of KIC in the medium: concentrations of 80 mg% KIC inhibited cell proliferation and concentrations of 120 mg% were sufficient to induce almost complete cell death within 30 h (Fig. 5). This effect was specific to KIC and was not observed with its related BCAA, leucine (Fig. 5). The highest concentration used in these experiments was 200 mg% (13 mM), because such concentrations were reported for BCAA and BCKA in blood tests of untreated MSUD patients [2,36], while in blood tests of healthy people, BCKAs were detected only in μM levels [2].

Significant cell death was observed as early as 2 h after addition of KIC to NIH3T3 cells. Such a rapid response suggests an active mechanism of cell death. To determine if KIC induces apoptosis in these cells we used FACS analysis and TUNEL staining. A summary of four FACS analyses of KIC treated cells is presented in Fig. 6A. A large sub-G1 population, characteristic of apoptotic cells, was found in KIC treated cultures. As shown in Fig. 6B, the TUNEL assay also detected apoptotic cells in KIC treated samples but not in untreated control cells. A large fraction of the treated cells (about 25%) was positively stained after incubation of only 4 h with KIC. The use of alkaline phosphatase for identifying apoptotic cells clearly shows that cells are intact when DNA fragmentation is already evident (Fig. 6B). This is a basic feature of apoptosis in contrast to other mechanisms of cell death. Taken together, the results show that KIC induces apoptosis in NIH3T3 cells when added in physiological concentrations.

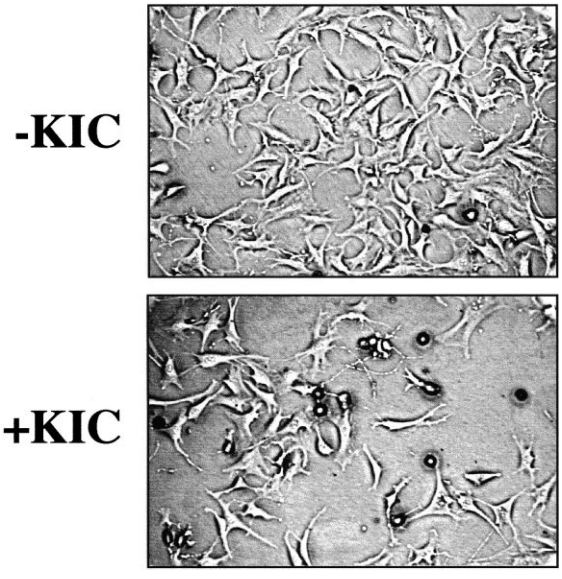

Fig. 4. Cells grown for 4 h with or without KIC (200 mg%). In the presence of KIC many cells are detached from the surface and appear more refractile.

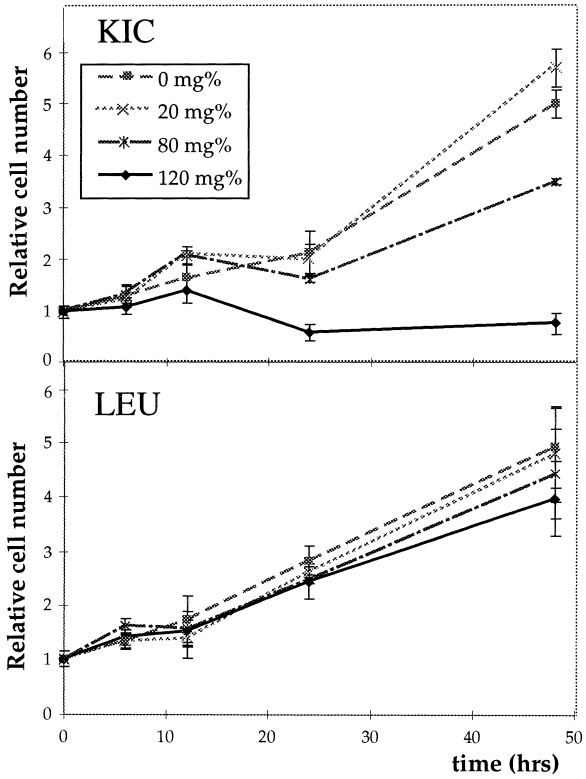

Fig. 5. Effect of KIC on growth of NIH3T3 cells. Growth of cells in 10% FCS with increasing concentrations of KIC (upper panel) or leucine (lower panel). Cell growth was followed for 48 h and estimated by a WST-1 assay. Each point represents three measurements (mean ± S.E.).

4. Discussion

c-Myc has diverse effects on cellular processes. It can promote cell proliferation as well as cell death, it can induce malignant transformation and is essential for embryonic development. Though the molecular mechanisms whereby these processes are exerted are relatively unknown, c-Myc probably promotes the various processes through the regulation of its target genes and in coordination with other factors present in cells. We set out to assess the role of *Bcat1/Eca39*, a target for c-Myc regulation, in cellular growth. Previous studies have indicated that disruption of the gene in yeast cells results in an accelerated growth rate [26], suggesting an anti-proliferative role for *Bcat1/Eca39*. In the current work we demonstrate a similar role for *Bcat1/Eca39* in mammalian cells as well: overexpression of *Bcat1/Eca39* inhibits cell growth and reduces cell viability (Fig. 2).

Serum deprivation directs cells into growth arrest but in most cases is not sufficient to induce programmed cell death.

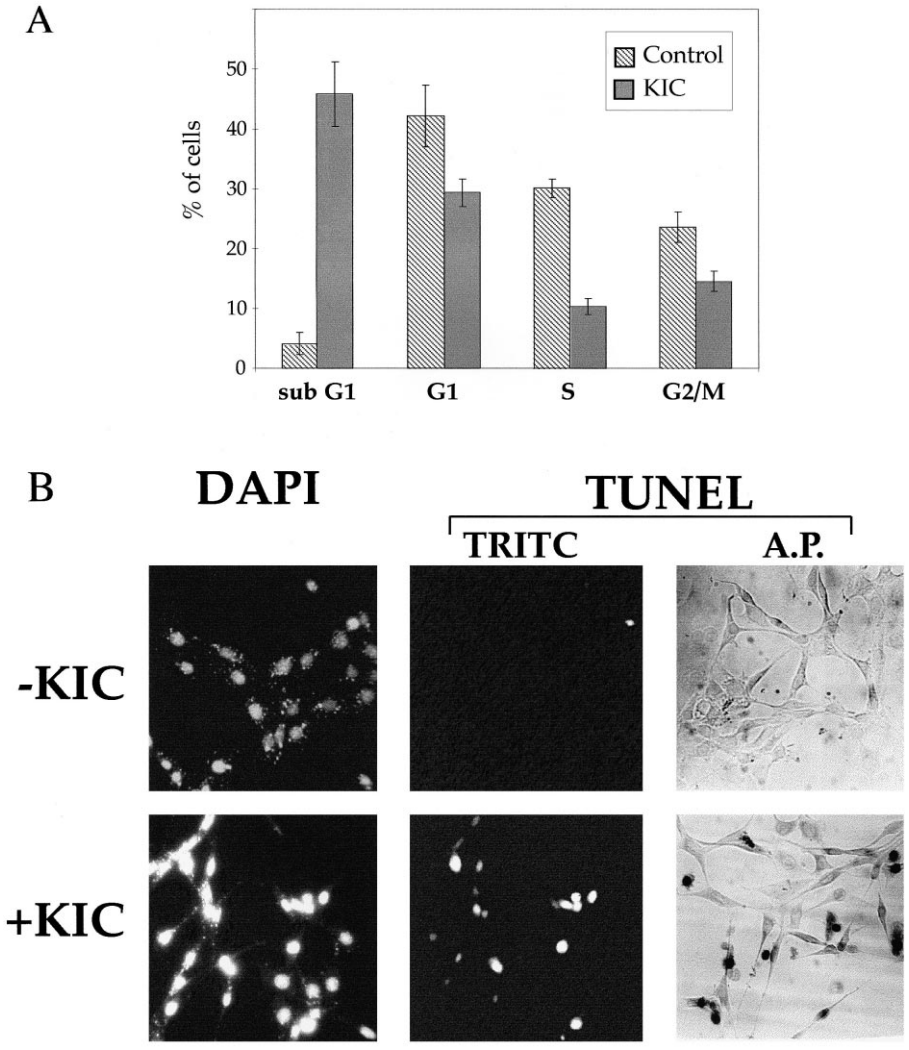

Fig. 6. A: NIH3T3 cells were grown with or without KIC (200 mg%) for 12 h. Cellular DNA content was determined using FACS analysis. The proportions of sub-G1 (apoptotic), G1, S and G2/M cells are shown. Each column represents data from four independent experiments (mean ± S.E.). B: TUNEL assay of cells grown with or without KIC (200 mg%) for 4 h. TUNEL was performed using TRITC or alkaline phosphatase for detection of labeled DNA. About 25% of cells were positively stained compared to less than 0.5% in the control. DAPI staining was used to detect nuclei and observe their morphology.

However, expression of c-*myc* in serum deprived cells can induce apoptosis [18]. Since *Bcat1*/*Eca39* was identified as a c-Myc target we asked whether the death of cells overexpressing *Bcat1*/*Eca39* is also the result of apoptosis. We therefore searched for apoptotic features in these cells. No single criterion exists for characterization of apoptotic cell death and not all apoptotic features are manifested in all cell types [34,37]. A common apoptotic feature is chromatin condensation followed by DNA fragmentation [37]. The results presented clearly show DNA fragmentation in intact cells detected by FACS and TUNEL analyses. DNA appeared degraded also when separated in agarose gel, though not in a ladder pattern (results not shown). Cell shrinkage and chromatin condensation were also observed (Fig. 3B), supporting the hypothesis of an apoptotic mechanism of cell death.

The anti-proliferative effect is evident in low serum conditions. This may indicate that the pathway is dependent also on environmental signals supplied by the serum. Another explanation is that because the endogenous *Bcat1*/*Eca39* is repressed only at low serum concentrations, the increase in expression due to the presence of the exogenous gene is not significant in high serum conditions and has no biological effect. In such a situation, disruption of the gene will decrease ECA39 activity in high serum conditions and reveal its effect on proliferation rate as observed in yeast cells [26].

Considering the recent finding that ECA39 is a branched-chain amino acid aminotransferase (BCAT) [4], it is unlikely that BCAT1/ECA39 directly regulates the cell cycle. It seems more probable that changes in the levels of metabolites involved in the transamination reaction or changes in the general metabolic state of the cell, mediate an indirect effect on cell division cycle progression. Two of the metabolites produced by BCAT have been previously proposed to affect cell growth: One is glutamate, a well characterized inducer of neuronal cell death via a specific receptor on the cell membrane [38], and the other is KIC which was shown to inhibit cell proliferation [36]. While glutamate levels in the cell are affected by several metabolic pathways, KIC levels are regulated directly by BCAT1/ECA39. Our results show that KIC can efficiently inhibit cell proliferation and can induce apop-

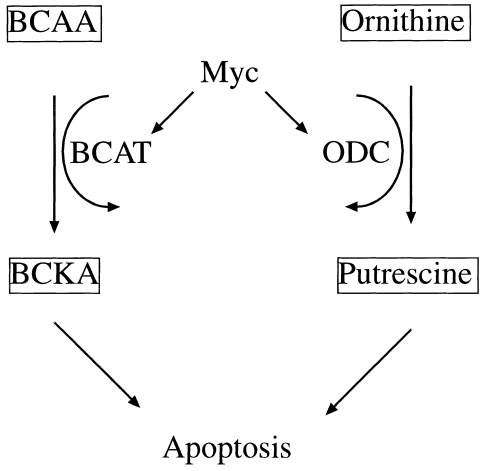

Fig. 7. Schematic representation of the effects of the Myc-induced metabolic enzymes BCAT and ODC on apoptosis. BCAA, branched-chain amino acids; BCKA, branched-chain keto acids.

tosis, when added at concentrations seen in MSUD patients. The apoptotic characteristics displayed by serum-starved cells overexpressing *Bcat1/Eca39* and by cells exposed to KIC are similar. The differences in the proportions of dead cells result from the different natures of the two systems: Unlike the death of *Bcat1/Eca39* overexpressing cells which is a more stochastic phenomenon, cells treated with KIC are exposed simultaneously and therefore respond more rapidly and uniformly. The anti-proliferative effect and the drastic apoptotic effect of high KIC levels on NIH3T3 cells offer a mechanistic explanation for the results obtained from overexpressing *Bcat1/Eca39* in mammalian cells. We propose that the effects of BCAT1/ECA39 on cell growth are mediated by KIC levels, which are regulated by BCAT1/ECA39. Overexpression of BCAT1/ECA39 increases KIC levels, inhibiting cell growth or promoting cell death. This model is in good agreement with results from yeast, in which disruption of *ECA39* accelerates growth [26] and addition of KIC inhibits growth (unpublished data).

BCAA are essential amino acids in mammals and are utilized for protein synthesis. Degradation of BCAA and production of BCKA occurs when energy sources are limited and protein catabolism becomes the energy source of the organism. It is appealing to suggest that under such conditions keto acids may serve as indicators for the metabolic state of the cells. Thus, high levels of BCKA may indicate a catabolic condition, producing an indirect suppressive effect on cell proliferation. Interestingly, a similar situation, in which amino acid metabolites normally found in cells in low concentrations induce cell death when overproduced, is found with another Myc target: Ornithine decarboxylase (ODC) (Fig. 7). ODC promotes the rate-limiting step in polyamine biosynthesis, converting ornithine to putrescine. Overexpression of *ODC* induces apoptosis in serum-starved cells [39], and ornithine itself is sufficient to induce apoptosis in mouse melanoma cells overproducing ODC [40], probably via accumulation of putrescine [40,41].

From the accumulating information on Myc target genes it is evident that Myc regulates the expression of genes involved directly in control of the cell cycle (e.g. *cdc25A*, *p53*) but is not restricted to cell cycle control. Myc regulates expression of various metabolic enzymes as well, e.g. ODC [42], BCAT1/ECA39 [7,9], carbamoyl-phosphate synthase (*cad*), [43], lactate dehydrogenase (LDH-A) [44], and these enzymes are also important mediators of Myc-induced processes [39,44].

Bcat1/Eca39 may be involved in yet another cellular process. As mentioned, transamination of BCAA produces glutamate in addition to BCKA. Glutamate is a widespread neurotransmitter, with cytotoxic effects. Glutamate levels and distribution in the brain are tightly regulated to allow production of proteins and neurotransmitters, and at the same time, prevent exposure of cells to the toxic effects of glutamate [45,46]. Transamination of BCAA, promoted by BCAT1/ECA39, is believed to be the main mechanism for balancing glutamate levels in the brain [45,46]. This would suggest that *Bcat1/Eca39* may play a role in brain development, Being a Myc target gene, *Bcat1/Eca39* may by related to Myc's essential role in development.

Our interest in Myc target genes is based on the assumption that understanding the mechanisms by which Myc transforms cells will facilitate the development of drugs specific for Myc-induced tumors. The realization that Myc oncoproteins are involved in apoptosis opens the field to try and shift the balance of cellular growth from proliferation to apoptosis in the tumor. The isolation of target genes for Myc activity, which are also involved in apoptosis, should assist us in the development of reagents and strategies to control tumor growth.

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