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REVIEW

MCL1 provides a window on the role of the BCL2 family in cell proliferation, differentiation and tumorigenesis

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SPOTLIGHT

The MCL1 gene (myeloid cell leukemia-1) was discovered serendipitously about a decade ago and proved to be a member of the emerging BCL2 gene family. Ongoing studies of this gene provide an interesting perspective on the role of the BCL2 family in transitions in cell phenotype. Specifically, gene products that influence cell viability as a major effect (eg MCL1, BCL2 and other family members) can act as key determinants in cell proliferation, differentiation and tumorigenesis. Although they do not have a direct role in proliferation/differentiation programs, these genes can either permit these programs to proceed or prevent them. Through such effects, the BCL2 family regulates the normal flow of cells through cycles of proliferation and along various pathways of differentiation. A model is presented suggesting that this is accomplished by sustaining or inhibiting viability at critical points in the cell lifecycle. These critical points represent windows of time during which cell fate transitions are effected. They can also be visualized as windows that open or close to promote or prevent continued progression along various cell fate pathways. The pattern of BCL2 family expression at these points allows for the proliferation differentiation, and continued viability of cell types that are needed, while aborting these processes for cells that are overabundant or no longer needed. The combined action of the various family members can therefore control the fate of cells, tissues and even the organism. This mechanism involving apoptosis-related genes is readily executable, and is poised to respond to external signals through the differential regulation of BCL2 family members. As such, it plays an important role in the maintenance of tissue homeostasis and function. Alterations that affect the BCL2 family impair the capacity to control the flow of cells through these critical points, and thereby 'leave the window open' for cell immortalization and cancer. Targeting this family may thus provide a means of inhibiting cancer development and inducing apoptosis in tumor cells.
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MCL1 was discovered based on increased expression during cell commitment to differentiation

MCL1 was originally identified as a gene up-regulated early in the differentiation of a human myeloid leukemia cell line, ML-1.[1] These cells proliferate at an immature myeloblastic stage and undergo terminal differentiation to non-proliferative monocyte/macrophages upon exposure to phorbol esters such as 12-0-tetradecanoylphorbol 13-acetate (TPA). Commitment to differentiation occurs rapidly – within a window of several hours – upon the application of TPA to growth-arrested cells.[2] The 'commitment window' precedes phenotypic differentiation, which proceeds over the next several days. ML-1 cells that have passed through the commitment window do not differ from the initial uncommitted population in their morphology or in the expression of cell surface differentiation markers. However, they are programmed to terminally differentiate and stop proliferating, as can be seen upon transfer to fresh, inducer-free medium.[2] MCL1 is expressed at peak levels in such committed cells (at 3 h), declining during their subsequent conversion to monocyte/macrophages and acquisition of differentiation markers such as CD11b and CD14 (see Figures 1 and 2 of Ref. 1). In contrast to MCL1, BCL2 is expressed at a constant level until decreasing as ML-1 cells reach a terminally differentiated stage.[3] An increase in MCL1 expression is seen in a variety of myeloid cell lines in response to various differentiation inducers.[1,3–7] A similar increase occurs during the retinoic acid-induced differentiation of embryonic stem cell and carcinoma cell lines.[8,9] Thus, from the outset, expression of MCL1 appeared to be linked to commitment to differentiation, suggesting a role at critical junctures in cell fate. Studies performed since that time expand upon this theme and suggest that MCL1 represents a prototype of the BCL2 family in that it acts during windows of transition in cell phenotype (eg changes in cell proliferation, differentiation, or viability).

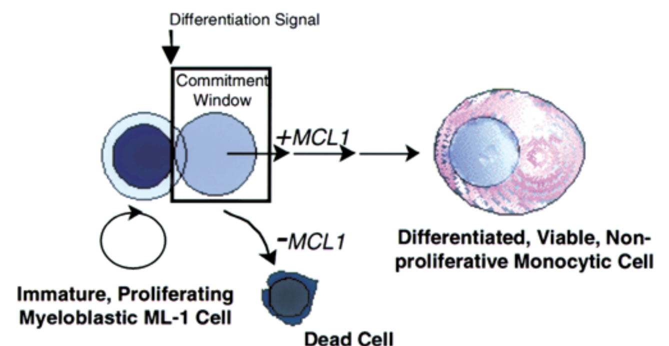

Figure 1 MCL1 provides a model of a BCL2 family member that acts at critical windows of transition in cell fate. In this model, modulation of viability-regulating gene products can control the flow of cells along a differentiation lineage: up-regulation of a viability-promoting gene product allows for an increase in the flow of cells along a particular lineage as needed, while down-regulation provides a rapid means of terminating this process. The BCL2 family member MCL1 was originally identified based on increased expression in ML-1 human myeloblastic leukemia cells that had committed to, but had not yet undergone, differentiation in response to the phorbol ester TPA. Peak expression of MCL1 occurs within 3 h after the application of TPA, while phenotypic differentiation occurs over days 1–3. This early increase in expression of MCL1 serves to promote the viability of myeloblasts initiating differentiation in response to other gene products. This allows cells to pass through the commitment window and further mature to monocyte/macrophages.

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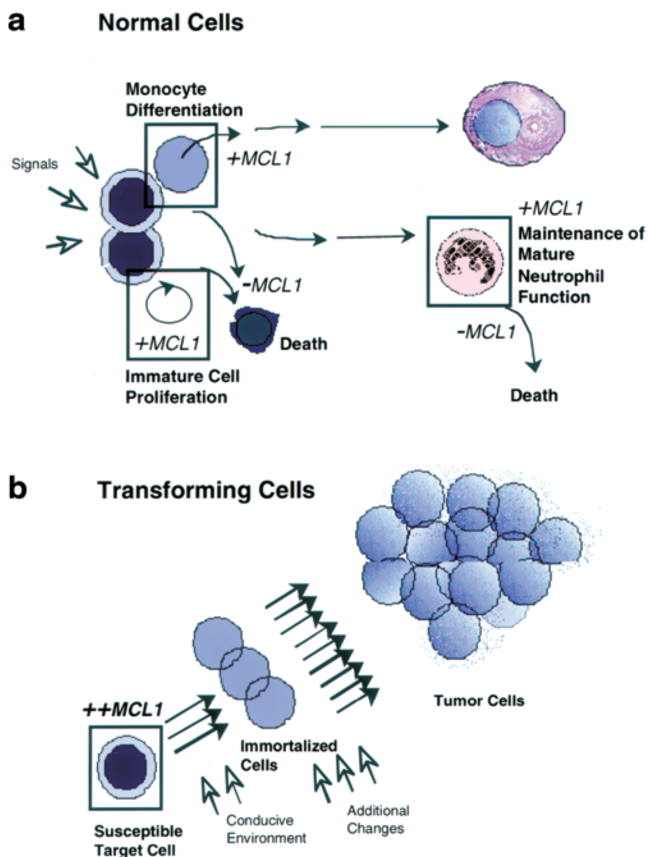

Figure 2 MCL1 normally acts at critical windows in cell proliferation, differentiation and viability, while alterations that affect its expression or activity 'leave the window open' to tumorigenesis. (a) MCL1 is proposed to act in critical phases or 'windows' during the lifecycle of normal cells. Examples of these windows include commitment to differentiation along the monocyte/macrophage lineage, stimulation of proliferation in progenitor cells, and the maintenance of viability and function in mature neutrophils. (b) Alterations in the normal tightly regulated pattern of MCL1 expression can result in extended cell survival. In a susceptible target cell type and a conducive growth environment, this can lead to cell immortalization and, with additional genetic changes, to tumorigenic conversion.

MCL1 promotes short-term viability in a variety of cell types

MCL1 promotes cell viability, as do BCL2, BCLX, and other anti-apoptotic members of this family. This can be seen in murine myeloid progenitor cells (FDC-P1), where transfection with MCL1 results in enhanced survival under apoptosis-inducing conditions.[10] This enhancement is seen upon the withdrawal of required growth factors, as well as upon exposure to a variety of cytotoxic chemotherapeutic agents. However, enhancement of survival is short-lived, as the MCL1-expressing cells live for several days longer than controls, but eventually die. A similar effect is seen in Chinese hamster ovary cells, where survival is not prolonged for as long with MCL1 as with BCL2.[11,12] Another hematopoietic progenitor cell line, 32D, does not exhibit enhanced survival with MCL1, but does with BCL2.[13] These early observations on the moderate effects of MCL1, along with its rapid inducibility, suggested a simple model for the physiologic role of MCL1: MCL1 might serve as a BCL2 family member that could be rapidly up-regulated during critical transitions in cell fate, its function being to provide short-term enhancement of cell viability.

The above model is exemplified by ML-1 cells initiating differentiation in response to TPA, where the increase in MCL1 expression appears to promote viability and allow cells to pass through the commitment window (Figure 1).[4,10] Accordingly, when antisense oligonucleotides or other approaches are used to prevent induction of MCL1 by TPA, cells die instead of undergoing differentiation.[14,15] In other words, increased MCL1 expression serves to provide additional protection of viability during commitment to differentiation. This may be needed because some of the other gene products up-regulated during the commitment window are potentially cytotoxic (eg cFOS, EGR1). Overall, the increase in MCL1 expression allows ML-1 cells to pass through the commitment window and continue along the monocytic differentiation pathway, whereas prevention of this increase aborts maturation because cells follow the alternate pathway to cell death. A similar role has been ascribed to MCL1 in the other types of differentiating cells, and to BCL2 in still others.[16–19]

In addition to transfected cell lines, transgenic mice have been generated that express an introduced human MCL1 transgene in hematopoietic tissues.[20] The MCL1 transgene was expressed at levels that are seen endogenously (eg upon stimulation with TPA), and was found to produce moderate viability enhancement.[20] Enhanced survival (several days to a week) was seen in mature myeloid and lymphoid (B and T) cells upon explantation into tissue culture, and was also seen in immature colony-forming cells. In addition, the transgenic animals exhibited moderate splenic enlargement (two- to three-fold), likely as a result of enhanced lymphocyte survival. In sum, MCL1 can produce moderate short-term viability enhancement in a broad range of cell types, including hematopoietic cells of various lineages at both immature and mature stages of differentiation. These observations in transgenic mice indicate that the above model, in which MCL1 promotes viability during transitions in cell phenotype, is applicable to a variety of other cells in addition to immature myeloid cells initiating differentiation.

During mouse development, MCL1 expression increases at early stages of embryogenesis (up to the morula/blastula stage), and is detected in embryonic tissue as well as in the surrounding maternal decidua.[21,22] Correspondingly, it has been reported that MCL1 knock-out mice do not live beyond the morula/blastula stage.[22] Thus, MCL1 is expressed during a critical window in early embryogenesis, and the absence of MCL1 may abort development beyond this point. These findings are also consistent with the above model, in which MCL1 plays an important role in transitions in cell phenotype.

MCL1 is normally expressed in a cell type-specific manner in response to specific signals that affect growth, differentiation and viability

As exemplified in early myeloid differentiation and embryogenesis, changes in MCL1 expression come into play in situations where cells are subject to a transition in phenotype. Summarized below are a growing number of examples of MCL1 expression during such transitions. These examples involve alterations in MCL1 expression during proliferation, differentiation, cellular stress and/or a change in viability. A theme that emerges is that MCL1 is exquisitely responsive to specific environmental signals, such as growth/differentiation factors and cellular stresses. Accordingly, MCL1 exhibits a

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pattern of expression that is highly regulated, and cell type- and differentiation stage-specific. In these characteristics, MCL1 typifies the viability-promoting BCL2 family members, since tissue surveys indicate that each of these has a unique pattern of expression and regulation.[23–27] Another emerging theme, also typical of these family members, is that increased expression of MCL1 is often associated with cell survival, while decreased expression is associated with cell death. A final theme is that expression of MCL1 is interwoven with that of other family members in complex patterns. Considered together, these themes are consistent with a broader model in which, not only MCL1, but the various members of the BCL2 family act in a coordinated network that allows multiple environmental signals to influence viability as cells pass through successive stages of proliferation and differentiation. In terms of the model being developed, this BCL2 family network can be envisioned as a series of windows that can be opened and closed to allow or prevent the implementation of cell fate decisions.

Differentiation stage specificity of MCL1 expression

The first, very vivid, demonstration of the cell type- and differentiation stage-specificity of MCL1 expression came out of immunohistochemical studies of its staining pattern in normal human tissues of hematopoietic, as well as non-hematopoietic origin.[24,27] These studies showed that MCL1 is expressed in immature cells of the myeloid and the erythroid lineages, but is not expressed in mature cells. The elevated levels of expression present at immature, but not mature stages of myeloid cell differentiation *in vivo* thus paralleled the observations from myeloid cell lines such as ML-1 undergoing differentiation *in vitro*.[1,4]

MCL1 is also expressed at specific stages in lymphoid cell differentiation, expression being low in naive B cells but prominent in germinal center B cells, and low in memory cells.[24,27,28] BCL2 exhibits the reciprocal expression pattern. Germinal center B cells are undergoing affinity maturation and the majority die by apoptosis, in contrast to naive and memory B cells which can live for an extended period. Expression of MCL1 in germinal centers may thus serve to promote viability for a brief window of time. This could allow for the selection of clones exhibiting appropriate antibody affinity, while at the same time ensuring the rapid elimination of the remaining cells. Germinal center B cells also express BCLX and, in the absence of growth factors, expression of BCLX and MCL1 declines and cell death ensues.[28,29] However, CD40 stimulation maintains BCLX and MCL1 expression and inhibits cell death. The interplay between several BCL2 family members thus influences B cell viability during passage through the germinal center and further differentiation. While the differential roles of MCL1 and BCLX remain to be elucidated, a speculative possibility is that MCL1 might primarily promote short-term germinal center cell survival to allow selection, while CD40-stimulation of BCLX sustains the viability of those cells selected for maturation rather than apoptosis. Outside germinal center cells, MCL1 is expressed in plasma cells and large activated lymphocytes.[24,27,29] Taken together, the above findings indicate that expression of MCL1 in human hematopoietic tissues occurs in specific cells at particular phases – or windows – in differentiation along various lineages. The normal *in vivo* pattern of expression thus accords with the 'windows' model of MCL1 action, derived from *in vitro* systems and mouse models.

Differentiation stage specific patterns of MCL1 expression also occur in non-hematopoietic tissues, further agreeing with the above model. A reciprocal pattern of expression of MCL1 *vs* BCL2 is seen in epithelial tissues, where MCL1 expression is low and BCL2 expression high in less differentiated, basal cells while the reverse is true in more differentiated cells in the upper layers.[27] This is exemplified in the skin, where MCL1 expression is weak in basal cells, increasing in the upper spinous and particularly the granular layers.[30] Here, prominent expression in cells that are differentiating and destined to die may relate to the short-term effects of MCL1 and to the fact the epithelial surface is subject to continuous turn-over and replacement. Overall, MCL1 and BCL2 exhibit distinct, highly regulated patterns of expression in various types of both hematopoietic and non-hematopoietic cells.

Tissue- and differentiation stage specificity of expression, which was not an unexpected finding for MCL1 as this was the basis of its identification, has proven to be typical of a variety of anti-apoptotic members of the BCL2 family. BCL2 and BCLX exhibit alternating expression during lymphoid development: while BCL2 (but not BCLX) is abundant at early stages (pro-B cells), BCLX is up-regulated and BCL2 down-regulated at intermediate stages (pre-B cells), and BCL2 is then re-expressed and BCLX down-regulated in mature cells (see Refs 23 and 31, shown schematically in Figure 5 of Ref 23). A comparable alternating expression pattern is seen during T cell development.[23] In sum, the various members of the BCL2 family exhibit distinct patterns of waxing and waning expression as cells of different lineages pass through successive stages of differentiation.

Induction of MCL1 by specific growth and differentiation signals

Differential expression of MCL1 along the differentiation continuum reflects the regulation of MCL1 by specific factors that affect cell growth, viability, and differentiation. In myeloid cells, growth factors such as interleukin-3 (IL-3) and granulocyte–macrophage colony-stimulating factor (GM-CSF) stimulate MCL1 expression and cell survival in progenitor cell lines dependent on these factors (eg TF-1).[5,15,32,33] The rapid increase in MCL1 mRNA, induced with either TPA or growth factors, does not require protein synthesis, as is typical of early response genes.[4,32] BCLX expression is also induced by hematopoietic growth factors (eg in 32D cells[34]). However, induction in this case is dependent upon protein synthesis. Furthermore, while the effects on MCL1 are transduced through the membrane distal region of the GM-CSF/IL-3 $beta_c$ receptor subunit, induction of BCLX requires the membrane proximal region.[32,35] A speculative possibility to encompass these findings is that different growth factor-induced signal transduction pathways result in protein synthesis-independent up-regulation of MCL1 for short-term promotion of cell viability, as well as protein synthesis-dependent up-regulation of BCLX for the maintenance of this response. These findings thus agree with the model of MCL1 as a BCL2 family member that can be rapidly induced for short-term viability enhancement. They further demonstrate that MCL1 can fulfill this role during the stimulation of proliferation, as well as during the induction of differentiation (Figure 2a) (see Ref. 36 for other effects of MCL1 on proliferation). Finally, the above findings illustrate coordination among BCL2 family members in the determination of cell fate.

Induction of MCL1 expression by growth factors can occur

in normal myeloid cells at both immature and mature stages of differentiation. At one end of the spectrum, MCL1 expression in immature CD34+ bone marrow cells is stimulated by factors that promote cell survival.[37] At the opposite end of the spectrum, MCL1 expression in mature neutrophils is maintained by factors that prolong their life span (eg GM-CSF, lipopolysaccharide (LPS), or hypoxia[38,39]). Neutrophils are terminally differentiated, non-proliferative cells, which are very short-lived. They provide an example of MCL1 expression that is associated with a switch in cell viability *vs* death in mature cells, rather than with changes in proliferation or differentiation (Figure 2a). The rapidly inducible, short-term effects of MCL1 may present an advantage for these cells: MCL1 may provide a means of extending viability when neutrophils are needed (eg in infection), while at the same time maintaining the capacity for rapid turn-over to prevent their potentially damaging effects (eg due to production of reactive oxygen species). As above, MCL1 acts in coordination with other members of the BCL2 family, since expression of anti-apoptotic A1 and pro-apoptotic BAX can also have effects on neutrophil viability.[40–44] Another principle that emerges from studies of neutrophils is that different cytokines can act through different BCL2 family members, since laminin adhesion-induced neutrophil survival does not appear to involve MCL1.[38,41,44] A similar principle arises out of studies in eosinophils, where the pattern of expression of MCL1 and other members of the BCL2 family appears to depend upon both the state of the cells and the type of cytokine applied.[45–49] In contrast to neutrophils and eosinophils, the application of survival signals to mature monocyte/macrophages results in increased expression of BCL2 and BCLX, but not MCL1.[50,51] In sum, the combination of BCL2 family members operating in the various windows along the differentiation continuum depends upon cell type and differentiation stage, as well as upon cytokine stimulation.

Just as MCL1 expression can be increased by factors that promote cell viability, it is often decreased by agents that cause cell death (eg sodium salicylate and etoposide[7,52]). It is not clear, however, whether such changes in MCL1 expression have a role in the loss of cell viability or occur instead as a result of this process. In other words, a decrease in MCL1 expression in dying cells might itself contribute to cell death, or might simply be a result of cell death and rapid MCL1 turn-over. In spite of this caveat, the above studies on MCL1 expression, taken together, are remarkable in the concordance of the results obtained from different systems. Furthermore, these studies on endogenous expression patterns reinforce those on exogenous gene transfer in demonstrating an association between expression of MCL1 and short-term viability enhancement.

As in the myeloid systems above, MCL1 expression in lymphoid cells is associated with the promotion of proliferation, differentiation and/or viability. In B cells, growth factor withdrawal results in decreased expression of MCL1 and cell death, while growth factor stimulation (eg with IL-4 or IL-13 plus CD40 ligand) has the opposite effect.[53,54] Activation of B or T cells also results in a rapid increase in expression of MCL1, along with a more sustained increase in BCLX and BCL2.[29] Signaling through the cAMP pathway has the opposite effect, selectively decreasing the expression of MCL1, but not BCL2, and enhancing cell death.[55] The response of B cells to cytokines can be modulated by other signals, as seen with IL-10.[56] If this cytokine is added as cells are exposed to an activating stimulus, expression of MCL1, BCL2 and BCLX decreases and cells undergo growth arrest and death. If IL-10 is instead added after activation, expression of these BCL2 family members is maintained, and the cells proliferate and differentiate.

A final example of MCL1 expression is seen in Epstein–Barr virus (EBV)-infected lymphoid cells. Increased expression of MCL1 occurs as an early event upon infection with the transforming viral protein, latent membrane protein-1 (LMP-1). Increased expression of BCL2 occurs as a subsequent event and may contribute to the immortalization of lymphoblastoid cell lines.[57–60] This appears to be another case where MCL1 can promote viability on a rapid short-term basis, which allows the recruitment of other family members for further cell fate decisions. MCL1 also has a role in the IL-6-induced differentiation of EBV-infected lymphoblastoid cells. Here, a pronounced increase in the expression of MCL1 (but not BCL2 or BCLX) occurs as cells differentiate towards plasma cell-like phenotype and stop proliferating.[61] This is followed by decreased MCL1 expression and cell death. IL-6 and other cytokines also induce expression of MCL1 in the malignant plasma cells present in multiple myeloma.[27,62–65] In summary, MCL1 expression is regulated by agents that stimulate proliferation, differentiation and viability in cells at various stages (‘windows’) along the lymphoid, as well as the myeloid lineage, increased expression being associated with cell survival.

MCL1 is also induced by exogenous stimuli in non-hematopoietic tissues. This is seen in the ovary, where gonadotrophins stimulate MCL1 expression and promote oocyte development.[66,67] This suggests that MCL1 might serve as a determinant of oocyte survival *vs* atresia, or might have a role in controlling the viability of normal *vs* defective oocytes. MCL1 might then have a role in fertility, as it has effects on ovulation, embryogenesis and placental development.[22,68] To summarize the findings from a variety of tissues, the ability of MCL1 to promote survival, along with its intriguing pattern of expression during transitions in proliferation, differentiation and viability, suggest a role in processes as diverse as hematopoiesis, the immune response and reproduction.

MCL1 expression as a response to certain cytotoxic stimuli

MCL1 expression is induced in response to certain cytotoxic stimuli, a seemingly paradoxical observation that was first noted in ML-1 cells exposed to microtubule disrupting agents such as colchicine.[4] Here, an increase in MCL1 occurs within 1–3 h, while cell death proceeds over several days. MCL1 was therefore proposed to serve as a rapid response gene promoting viability for a limited period after exposure to the potentially cytotoxic agent. This possibility was consistent with studies with inducible MCL1 constructs, which showed that MCL1 promotes viability when induced either before or within several hours after the application of an apoptosis-inducing stimulus.[10] Thus, up-regulation of MCL1 by microtubule disrupting agents could represent a stopgap measure, allowing a window of time for cells to either up-regulate other viability-promoting family members or, alternatively, initiate apoptosis. Rapid up-regulation of MCL1 as a short-term mechanism for maintaining viability is supported by the finding that inhibition of colchicine-induced increase in MCL1 expression results in a striking acceleration of cell death.[15]

MCL1 expression is similarly induced in response to certain types of genotoxic stress, such as radiation. This increase is seen only in cells sensitive to radiation-induced cytotoxicity, suggesting that cell stress or damage serves as the signal.[69,70]

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Expression of BAX and BCLX is likewise rapidly induced by genotoxic stress, while expression of BCL2 is slowly decreased.[69,71–73] The radiation-induced increase in MCL1 expression is not dependent on p53, as assessed in p53 wild-type *vs* mutant or nul cell lines, in contrast to the effects on other family members.[69,71–73] In this instance as in others above, MCL1 may serve for rapidly inducible, short-term viability promotion, with other BCL2 family members participating in further decisions in cell fate.

Induction of MCL1 as a rapid response to stress is also seen in non-hematopoietic cells. In cardiac muscle, expression of MCL1 and cFOS increases early after myocardial infarction.[74] While cFOS is expressed in both ischemic and non-ischemic tissue, MCL1 is expressed only in non-ischemic tissue, consistent with a role in cell viability. MCL1 expression is also associated with cardiac myocyte viability in the presence of atrial natrietic factor.[75] Finally, MCL1 expression is increased in the skeletal muscle of mice lacking the mitochondrial adenine nucleotide transporter, possibly as a result of increased oxidative stress.[76] In short, MCL1 can be rapidly up-regulated in response to diverse cellular stresses, in addition to signals for proliferation, differentiation and viability.

MCL1 contributes to long-term immortalization and tumorigenesis in susceptible cell types placed in a conducive environment

While moderate effects are seen with MCL1 in short-term viability assays, dramatic results have been obtained in long-term studies. This was first seen when hematopoietic cells from MCL1 transgenic mice were cultured in the presence of IL-3. Maintenance of these cultures for ~1–2 months invariably resulted in the outgrowth of immortalized cell lines, an effect never seen in parallel nontransgenic control cultures.[20] Two types of cell lines were obtained, immature mast cells and monocytes (derived by maintaining nonadherent *vs* adherent cells), and both of these were dependent on IL-3. Immortalization therefore appeared to depend upon the expression of MCL1 in a suitable target cell placed in a conducive growth environment. Immortalization has not been reported with BCL2 by itself, but is seen with BCL2 plus MYC.[77] The IL-3 required in the case of immortalization with MCL1 may thus serve to stimulate proliferation in a manner analogous to the introduction of MYC in the case of BCL2.[78] Immortalization is one component of tumorigenic conversion.[79] Therefore, these findings with MCL1 suggested that expression of a BCL2-like gene, in an appropriate cellular and environmental context, may be capable of contributing to the eventual development of cancer. To further develop the analogy, these gene products may ‘open the window’ to tumorigenic conversion.

Pronounced long-term effects have recently been observed in MCL1 transgenic mice monitored for up to 2 years. These mice have a high probability of developing B cell lymphoma (>80%[80]), which appears with long latency and exhibits additional clonal genetic changes as seen with BCL2.[81] Several events probably contribute to these long-term effects. These include (1) the enforced expression of MCL1 at levels known to promote cell viability; (2) the endogenous constellation of gene products expressed in the target cells; (3) other gene products affected by growth/environmental factors; and (4) gene products altered by additional genetic changes occurring in the clone undergoing tumorigenesis. The above observations allow us to expand upon the model being developed as follows. MCL1 may normally serve to enhance short-term survival during specific windows in the cell lifecycle; however, enforced expression, in a susceptible cell type and conducive environment, can enhance viability over the long term and thereby open the window for immortalization and additional genetic changes that lead to tumorigenesis (Figure 2b).

MCL1 is regulated through multiple transcriptional and post-transcriptional mechanisms

MCL1 is regulated at the transcriptional, post-transcriptional, and post-translational levels. Induction by TPA in hematopoietic cell lines involves increased transcription,[4] which is stimulated through an extracellular signal-regulated kinase (ERK)-mediated pathway.[15] This pathway results in the activation of a transcription factor complex that contains serum response factor (SRF) and Elk-1 and that is bound to the human MCL1 promoter upstream of the start site of transcription.[82] MAP kinase mediated pathways (either MEK/ERK or p38) are also involved in the induction of MCL1 expression in other systems.[83–85] An additional pathway, transduced through phosphatidylinositol 3-kinase (PI3K) and AKT, is involved in the induction of MCL1 expression by GM-CSF and IL-3 in hematopoietic progenitor cells. This latter pathway acts through a transcription factor complex that contains cAMP response element binding protein (CREB).[86,87] The MEK/ERK and PI3K/AKT pathways can act coordinately in some cases.[33] Yet a third pathway involving JAK and STAT3 is stimulated by IL-6 and other agents in myeloma cells and by GM-CSF in neutrophils.[62,88–90] Altogether, several pathways, including MAP kinases, PI3K/AKT and JAK/STAT, can stimulate MCL1 transcription (Table 1), which probably contributes to its highly regulated pattern of expression.

MCL1 can also be regulated post-transcriptionally through alternative splicing. While full length MCL1 consists of three

Table 1 Signal transduction pathways involved in the stimulation of MCL-1 expression

Signal transduction pathway	*Cell system*
MEK/ERK	• TPA-induced immature myeloid cells (ML-1) (constitutively bound SRF/ETS activated)[15,82] • EGF-induced esophageal carcinoma cells (CE81T/VGH)[85] • Pancreatic carcinoma cells (PaCa-2)[84]
P38 MAPK	• Hypoxia-induced human neutrophils[83]
PI3K/AKT	• IL-3 or GM-CSF-induced TF-1 hematopoietic progenitor cells and BAF/3 pro-B cells (inducibly bound CREB activated)[87] • IL-6-induced Hep3B cells[86]
PI3K/AKT and MEK/ERK[a]	• IL-5, SCF-induced TF-1 cells[33] • GM-CSF-induced TF-1 cells (MEK/ERK pathway affects transcription while PI3K/AKT affects MCL1 protein translation)[134]
JAK/STAT	• IL-6 or IFN-induced human myeloma cells[62,88] • Large granular lymphocytes[89]
JAK/STAT and PI3K[a]	• GM-CSF-induced human neutrophils[90]

[a]Both pathways can have effects.

coding exons, a splice variant $MCL1_{s/\Delta TM}$ arises by juxtaposition of the first and third exons.[91] Full length MCL1 contains the BH1-BH4 BCL2 Homology domains, but the variant contains only BH3. Like the BH3-only members of the BCL2 family, the $MCL1_{s/\Delta TM}$ splice variant induces cell death rather than promoting viability.[91,92] Hence, MCL1 can be converted by alternative splicing from an anti- to a pro-apoptotic gene product, as can BCLX.[93]

Finally, MCL1 is subject to post-translational modification by phosphorylation.[94] Two types of MCL1 phosphorylation have been observed, which are effected through different pathways. One of these pathways is stimulated by TPA and depends on ERK activation. The second pathway is stimulated by agents that cause accumulation in G_2/M phase of the cell cycle (eg colchicine, taxol) or inhibition of protein phosphatase 1/2A (eg okadaic acid). Only the phosphorylation induced through the second pathway results in an electrophoretic mobility shift (band-shift) of the MCL1 protein. Phosphorylation of BCL2 has been suggested to either enhance or inhibit its viability-promoting function.[95–102] Interestingly, TPA-induced ERK-dependent MCL1 phosphorylation occurs in viable cells, while the taxol- or okadaic acid-induced MCL1 phosphorylation/band shift occurs in cells that are dying. Viewed as a whole, these findings suggest that several phosphorylation pathways may have differing effects on MCL1, BCL2, and possibly other anti-apoptotic family members. MCL1 is also a rapidly turned over protein containing PEST sequences and appears to be regulated through effects on protein stability like BCL2.[3,103–105] The conclusion emerging from these ongoing studies is that MCL1 is highly regulated at multiple levels. The ability to closely regulate the expression and activity of this gene product may serve as a means of finely controlling cell fate decisions, allowing for the production of appropriate numbers of required cells while preventing excessive accumulation and allowing elimination of cells that are no longer needed. Multiple levels of regulation are probably also important for avoiding the possibility of cell immortalization and tumorigenic conversion.

The BCL2 family plays a role in human cancer and may provide targets for therapy

As described above, the regulated expression of MCL1, BCL2, and other family members promotes viability as normal cells move along the continuum of hematolymphoid cell maturation. Alterations in this highly regulated pattern of expression can contribute to cancer. In fact, BCL2 was first identified at the t(14;18) chromosome translocation breakpoint characteristic of follicular lymphoma, and BCL2 is highly expressed in other cancers such as chronic B cell lymphocytic leukemia (CLL) and diffuse large cell lymphoma.[106–109] Recent findings suggest that, beyond the alteration in BCL2, follicular lymphoma involves an imbalance between anti- *vs* pro-apoptotic BCL2 family members.[28] Specifically, the lymphoma cells differ from normal germinal center cells both in the expression of multiple anti-apoptotic family members (BCL2, BCLX, and MCL1) and in the lack of expression of pro-apoptotic BAX and BAD. In other words, multiple BCL2 family members play a role in the genesis of this disease.

The expression of BCL2 family members in patient cancer cells might serve as useful indicator of disease responsiveness. For example, in B-CLL, an elevated BCL2/BAX ratio and elevated MCL1 expression are associated with progressive disease, decreased responsiveness to therapy, and shorter survival.[107,110,111] Similarly, treatment of B-CLL cells *in vitro* with chemotherapeutic agents that inhibit protein kinases results in decreased MCL1 expression and cell death.[112,113] The profile of BCL2 family expression may also serve as a marker of relapse, as suggested by a study of acute leukemia where expression of MCL1 (but not BCL2 or BCLX) was frequently increased upon disease recurrence.[114]

Beyond serving as a predictor of response to currently available therapeutic agents, a new approach to the treatment of cancer involves developing the ability to target BCL2 family members.[115] With this type of strategy, cancer cells could be monitored for their profile of BCL2 family expression. Approaches could then be developed to turn off or block the effects of anti-apoptotic gene products that are inappropriately up-regulated in the cancer cells (eg through the use of antisense oligonucleotides or small molecules[115,116]). Additional approaches could be aimed at promoting their alternative splicing to pro-apoptotic forms,[91,109] altering their activity through post-translational modification,[94] or at identifying stimuli that turn on pro-apoptotic gene products.

The windows model developed above has implications for the possibility of targeting the BCL2 family. The fact that anti-apoptotic family members are postulated to act at specific points during the differentiation of normal cells, while being expressed in a deregulated or constitutive manner in cancer, suggests that these targets have some selectivity. Thus, therapy could be directed against BCL2 family members that are critical to the sustained viability of tumor cells, but that do not play a key role in the survival of important types of normal cells. While some normal populations would be affected, cytotoxicity would be expected to be largely restricted to cells within particular windows of differentiation. Ideal targets would be BCL2 family members that are highly expressed in certain types of cancer cells, but not in normal stem cells. For example, BCL2 is expressed abundantly in many cases of AML, as well as various types of lymphoma, but not normal hematopoietic stem cells. Inhibition of the expression or effects of BCL2 might then be capable of inducing apoptosis in the leukemia/lymphoma cells, while sparing normal cells at early stages of differentiation. Although certain populations of normal cells would likely also be affected (eg B cells expressing high levels of BCL2[23]), cells at early stages of differentiation would be spared, allowing for the repopulation of depleted cell pools. After the administration of therapy aimed at inhibiting BCL2 family members expressed in the tumor cells, it might be possible to administer growth factors to expedite the re-expansion of normal lineages that have been affected. Alternatively, agents targeting BCL2 family members expressed in the tumor cells could be co-administered along with growth factors known to up-regulate other anti-apoptotic BCL2 family members in normal stem cell populations.

Several factors present an advantage for therapy directed against anti-apoptotic BCL2 family members. Thus, while several anti-apoptotic family members are often expressed in tumor cells,[28] inhibition of one of these can induce apoptosis despite the continued expression of the others.[116] This probably relates to the fact that the overall effect of the BCL2 family depends upon the balance between anti- and pro-apoptotic family members, where inhibition of one anti-apoptotic member can tip the balance towards apoptosis.[92,117] Another factor is that tumor cells often exhibit increased expression of proliferation-stimulating gene products. Such gene products can have apoptosis-inducing effects, as seen in the well studied example of c-Myc.[118] Tumor cell viability in this case is dependent upon the expression of anti-apoptotic BCL2 family

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members.[78,118] Inhibition of these family members would therefore allow the expression of the pro-apoptotic properties of other gene products endogenously expressed within tumors. Overall, therapy directed against the BCL2 family could be tailored to target specific family members expressed in individual patient tumors, and could gain further selectivity by taking advantage of the expression in these tumors of potentially pro-apoptotic molecules.

The above approach will entail further development of means of targeting the BCL2 family. It will also depend upon the refinement of assays for monitoring BCL2 family expression in patient tumor samples (eg by flow cytometry). Finally, it will be important to gain background information on BCL2 family expression patterns in normal cells, particularly in critical populations such as hematopoietic stem cells, crypt cells in the gastrointestinal tract and various types of immune effector cells. Means of targeting anti-apoptotic BCL2 family members are under intensive investigation. A promising avenue of current investigation is the use of antisense approaches, such as antisense oligonucleotides.[119] This approach has been shown to induce apoptosis in a variety of different types of tumor cells, including lymphoma, breast cancer, prostate cancer, renal cell carcinoma, malignant melanoma and malignant glioma.[120–126] This approach can also be used in combination with cytotoxic chemotherapeutic agents, resulting in enhanced tumor cell death.[109,120,123,127] A recent creative approach involves the use of bispecific oligonucleotides that target a sequence in common in BCL2 and BCLX.[128,129] Additional research is examining means of delivering antisense reagents, such as by targeting oligonucleotides to the cancer cells (eg through conjugation to peptides that bind to the surface of the tumor, or the use of liposomes or antisense ribozymes in adenovirus vectors[116,130,131]). Since some family members, including BCLX and MCL1, can be alternately spliced to yield pro- instead of anti-apoptotic gene products, yet another approach is to use antisense oligonucleotides that favor this alternate splicing (eg antisense oligonucleotides that match a splice junction necessary for splicing of the anti-apoptotic gene product[109]). Since anti-apoptotic BCL2 family members are also subject to post-translational modification through multiple pathways, such as phosphorylation, it may be possible to develop means of converting them to death-inducing forms. The attraction of the latter two approaches (alternate splicing and post-translational regulation) is that they are aimed at converting a molecule that is highly expressed in the tumor cells to a death-inducing product.

Summary

Findings with MCL1 suggest a model in which the members of the BCL2 family, through effects on viability, influence the overall pattern of cell proliferation and differentiation as well as the life span of functional, mature cells. In this model, BCL2 family members are envisioned to regulate the flow of cells through various proliferation and differentiation pathways, serving as determinants in the decision as to which cell types will be maintained or increased in number *vs* which types will be turned over, reduced in number, or eliminated. This action is not carried out through effects on proliferation and differentiation programs *per se*,[132] although it is carried out in coordination with these programs. Instead, the BCL2 family is postulated to promote or inhibit cell viability at critical steps of these processes. Unique combinations of BCL2 family members, acting within progressive windows along the differentiation continuum, may allow cells to pass through successive viability checkpoints and move towards a particular differentiated phenotype. The pattern of BCL2 family expression may act in a similar fashion in cells stimulated with growth factors to initiate the cell cycle and proliferate, allowing for amplification of the differentiating cells. One advantage of this mechanism utilizing viability-regulating gene products is that it could aid in producing a rapid, as well as a sustained increase in particular types of cells (eg in wound healing, infection, inflammation, or an immune response). This is because enhancing the survival of cells nearing the completion of the differentiation sequence could provide for a rapid increase in mature cells. At the same time, enhancing the survival of cells at earlier stages of the pathway would allow for replenishment and a sustained response. A second advantage of this mechanism is that it can be readily down-modulated when the desired number of cells is reached, when a particular type of cell is no longer needed, or when cells become old, damaged, or overabundant. Closing the viability window through effects on the BCL2 family would provide a means of rapidly stopping the flow of cells along a particular pathway without necessitating complex reprogramming of proliferation or differentiation commands, since these commands are simply prevented from being effected. This mechanism may be particularly efficacious because cells are postulated to have endogenous effector pathways poised to induce cell death.[133] A final advantage is that this mechanism of regulation by the BCL2 family provides the capability of responding in a selective fashion to a host of growth and differentiation signals, which can differentially affect various family members in a cell type-specific manner. This can compound the rapid responsiveness and flexibility of the system. It allows specific signals to target cells of defined maturation states, with additional signals being transmitted in parallel to coordinately influence cells at other stages of maturation or of other lineages. These signals can be rapidly countermanded as this became necessary, the final goal being to attain a coordinated, finely tuned physiologic outcome. This mechanism, linking the control of cell viability to that of proliferation and differentiation, is thus likely to play an important role in the maintenance of tissue homeostasis and function in multicellular organisms.

To control normal transitions in cell phenotype as described above, the activity of the BCL2 family must be closely regulated. Restated in terms of the analogy presented here, the ‘windows’ of BCL2 family activity must readily open and close in response to appropriate signals. Aberrations in this highly regulated activity, such as the deregulated overexpression of BCL2 seen in lymphoma, can then be considered as being akin to having a window ‘stuck’ in an open position. When this occurs, a means of stemming the flow of cells along the affected pathway is lost. With the window locked in the open position, cells may remain viable and enter the commitment window for a particular differentiation pathway whether this type of cell is needed or not. A window locked in an open position may similarly result in the loss of a check on the proliferative cycle, abrogating a means of modulating the effects of proliferative stimuli. The extended survival of this target cell population may, in a conducive environment, result in a very long-lived or immortal cell population. This population in turn is at risk for additional genetic changes, and has a high probability of eventual tumorigenic conversion. A means of closing or barricading the open window by targeting the BCL2 family

might then aid in preventing the development of cancer and in triggering apoptosis in cancer cells.

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