

Negative Control of Cell Proliferation. Growth Arrest Versus Apoptosis. Role of β GBP

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(Received 7 January 1997; In final form 9 June 1997)

β GBP is a novel physiological negative growth regulator of the cell cycle and a cytostatic factor. It is secreted by cells and acts by binding with high affinity to specific cell surface receptors. In normal cells, β GBP physiologically controls transition from G_0 to G_1 and passage from late S phase to G_2 by modulating signalling cascades activated by tyrosine kinase receptors and by affecting transcription events. As a cytostatic factor β GBP has a marked growth inhibitory effect on a variety of tumours including leukaemias where growth arrest is followed by the activation of apoptotic pathways and cell death.

Keywords: β GBP, cytokines, negative cell cycle regulation, checkpoints, apoptosis

The control of cell number within a given lineage is determined by a balance between cell proliferation and apoptosis, a genetically encoded death programme defined by characteristic biological and biochemical changes [1-4 and 5-7 for reviews]. Cell proliferation is a function of the cells' ability to enter and progress through the cell cycle, a highly conserved sequence of events by which eukaryotic cells replicate [8].

Entry into the cell cycle demands the activation by growth factors of signalling cascades which set into motion a tightly controlled interplay between growth promoting genes and cell cycle controller genes whose operational result is a timed progression through the cell cycle and the successful alternation of DNA synthesis and mitosis (see ref. 8 for overview and related terminology). Such an accurate control is articulated at level of checkpoints,

times within the cell cycle when programmed pauses secure that each cell cycle phase is completed before the cell progresses into the next [9,10]. Conditions which impair the cell cycle or the integrity of the DNA may impose a shift from cell proliferation to cell death. Thus both the altered expression of growth promoting genes and of cell regulatory genes which limit and modulate cell cycle progression, can induce cell death [6,7].

ONCOGENES, CELL CYCLE AND CELL DEATH

Evidence that oncogenic lesions that promote cell proliferation can be a cause of apoptosis [11,12] is best provided by studies on the *c-Myc* protein [13-15]. Ectopic activation of the proto-oncogene

c-myc in G_0 [13], a time when no cell cycle events are in act, and *c-myc* activation after S/G_2 commitment, also an ectopic expression, can trigger the onset of apoptosis [15].

Apoptotic death linked to functional alterations of cell cycle regulatory proteins [8] is documented in numerous instances: premature activation of the mitotic kinase p34 *cdc2* is associated with T cell induced apoptosis [16]; lack of functional p105^{rb} protein, a tumour suppressor protein which modulates the activity of the E2F-1 transcription factor at G_1/S transition is a cause of apoptosis [17–21]; also a lack of suppression of E2F-1 DNA binding by cyclin A cdk2 kinase, a key cell cycle regulatory complex, in the course of S phase can lead to S phase delay/arrest and apoptosis [22]. In both these latter instances cell death is conceivably the result of unscheduled E2F-1 DNA binding, perturbation of the process of DNA replication, unrepaired DNA and accumulation of DNA damage. Cyclin A, *cdc2* and *cdk2* are also implicated in apoptosis induced by agents which damage the cell genome [23–26]. However genotoxic agents, including anti-cancer drugs, may fail to induce apoptosis in cells where the p53 tumour suppressor protein, which

can transduce genome damage into growth arrest or apoptosis, is functionally absent [27].

CELL CYCLE CONTROL BY NEGATIVE GROWTH FACTORS

Some of the conditions outlined above indicate that cell death may result from an unscheduled forward drive in a cell as yet unprepared to enter a new cell cycle phase. Such a conflict between signals to proceed and unpreparedness to proceed may conceivably take place at the level of checkpoints. Which molecular events associate with checkpoint control is still open to analysis but there is evidence that regulatory messages to the effector machinery of distinct cell cycle stages and specific checkpoints originate from signalling pathways activated by negative growth factors that animal cells constitutively produce (Figure 1). Thus the cell cycle regulatory effect of the α/β interferons (IFNS), which negatively control G_1 transition, has been mapped to the second half of G_1 [28] and TGF β (transforming growth factor β), also a negative regulator, can cause late G_1 arrest [29,30]. More recently G_0/G_1

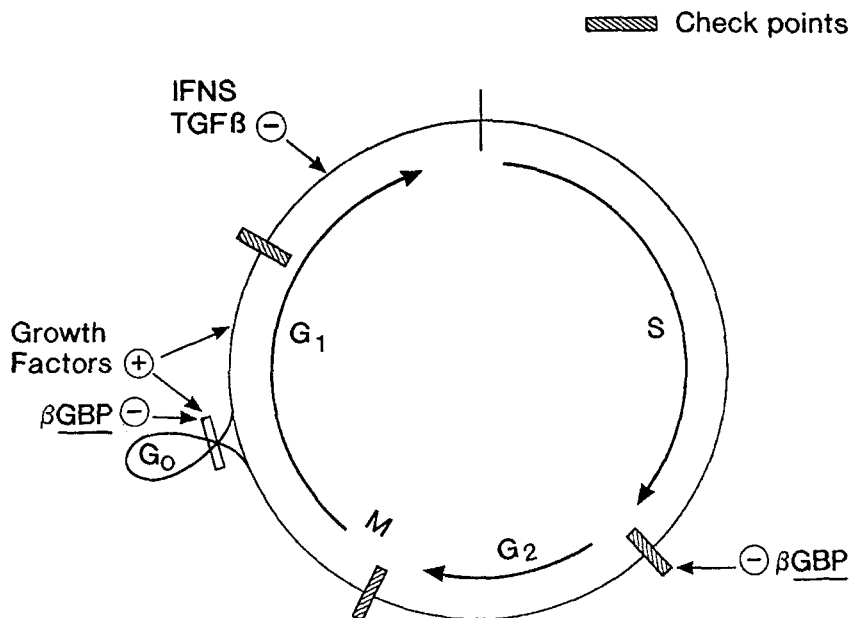


FIGURE 1 Positive and negative growth factors, cell cycle stages and checkpoint controls.

and S/G₂ transition have been found to be under the control of β GBP [31–34]. β GBP (β galactoside binding protein) is a monomeric protein of 134 amino acids encoded by a single gene [32] which maps on chromosome mu 15E/Hu 22q12-q13 [34] in the SIS/PDGFB homology region a syntenic group which undergoes deletion and translocation in a number of human tumours. Secreted by cells β GBP binds to some 5×10^4 cell surface receptors with a Kd of 10^{-10} M. As a physiological endogenous regulator of the cell cycle β GBP modulates transition from G₀ to G₁ and traverse from late S phase into G₂ by exerting controlled restraints. Negation of the constitutive endogenous β GBP by neutralising monoclonal antibodies anticipates entry into G₁ and accelerates traverse into G₂. As a cytostatic factor β GBP prevents exit from G₀ and magnifies checkpoint restraints by halting proliferating cells at the threshold of G₂ [31].

Control of Cell Cycle Entry by β GBP

Current investigations in our laboratory on G₀/G₁ transition control show that β GBP modulates transducing events along the MAP kinase pathway [35 for review] initiated from tyrosine kinase receptors and that the effect is downstream of receptor and upstream of Raf-1. β GBP inhibits the expression of the early growth promoting genes Fos and Jun in response to EGF (epidermal growth factor) and other polypeptide factors which activate growth and the expression of the *c-myc* proto-oncogene. p42 and p44 MAP kinases are not phosphorylated, while cyclic AMP levels remain unaltered, but EGF receptor phosphorylation does occur. By contrast β GBP does not prevent phosphorylation of p42 and p44 MAP kinase induced by phorbol myristate acetate (PMA) which acts via protein kinase C to Raf-1. Thus in the presence of β GBP, polypeptide growth factors fail to activate signalling cascades and early growth promoting genes, a fact which can conceivably be regarded as an equivalent of growth factor withdrawal. This is a condition which can lead to apoptosis when a cell is forced to pass the threshold from the quiescent G₀ state to G₁ by unscheduled

ectopic *c-Myc* expression [13] while still unprepared to enter the cell cycle.

S/G₂ Checkpoint Control by β GBP

Successful cell proliferation requires DNA integrity and accomplished DNA replication. Which monitoring system(s) may guard DNA integrity during progression within S phase is not defined, but a cell cycle pause at the end of S phase would secure completion of DNA replication before commitment to mitosis. The following evidence indicates that a late S/G₂ checkpoint is under the control of β GBP: neutralisation of the autogenous β GBP accelerates traverse through G₂; addition of exogenous β GBP forces cells to arrest at the S/G₂ threshold; passage through G₂ and mitosis follows the removal of the exogenous β GBP [31].

Current molecular analysis in our laboratory on cell cycle regulatory molecules during S phase shows that when normal proliferating fibroblastic cells of recent embryonic derivation are arrested by β GBP the programme of changes in E2F-1 and E2F-1 complexes is altered. In the presence of β GBP, free E2F-1 levels, which in the untreated cells increase from G₁ to mid S phase and descend progressively thereafter, remain on a plateau and E2F-1 complexes undergo no apparent changes as cells approach G₂. This is of particular importance in view of recent analysis with wild type E2F-1 and mutant derivatives defective in cyclin A kinase binding demonstrating that in NIH 3T3 cells orderly S phase progression is linked to the formation of stable E2F-1/cyclin A/cdk2 complexes and that the unscheduled presence of free E2F-1 on specific DNA sequences during S phase can activate a specific S phase checkpoint and cause cell cycle arrest, accumulation of cells in S phase and apoptosis [22].

GROWTH ARREST VERSUS APOPTOSIS

Recently we have examined the effect of β GBP on human leukaemic T cells representative of different stages of maturation and found that growth

arrest at S/G₂ is followed by the gradual activation of apoptotic pathways and cell death. If β GBP is removed prior to the activation of these pathways, tumour cells do not proceed into apoptosis. The molecular players involved in the induction of apoptosis by β GBP include members of the Bcl 2 family [36–39] and members of the tumour necrosis factor family [40] but there is no involvement of *c-myc* and of the p53 tumour suppressor gene, a fact possibly in accord with the cells being arrested at the late S/G₂ stage of the cell cycle. The fact that β GBP induced cell death in tumour cells does not involve the p53 gene is of some interest as it suggests that β GBP may be able to force p53 defective cells into apoptosis. Importantly the effect of β GBP in mitogen activated human T lymphocytes from healthy donors cultured in the presence of IL2 is limited to an S/G₂ cell cycle pause. Thus normal cells exposed to β GBP do not undergo apoptosis, they resume growth. This differential, selective response to β GBP could conceivably be explained by an imbalance between a strong mitogenic drive in tumour cells and their unpreparedness to undergo division due to a not totally completed S phase and faults in DNA repair during cell arrest at an S/G₂ checkpoint barrier. This is in accord with mounting evidence that events which interfere with DNA replication and checkpoint control, and which can thus impair the cell cycle or the integrity of DNA, can cause cell death in tumour cells but not in normal cells [27,41].

Our results on the selective action of β GBP, which induces apoptosis in leukaemic T cells but not in normal T lymphocytes, provides further evidence that treatments which induce apoptosis in tumour cells fail to do so in the normal cell counterparts [27,41] and suggests that β GBP may have a potential role in cancer surveillance and cancer control.

PROSPECTS

A diversity of extra and intracellular factors involved in the regulation of gene expression, DNA replication and cell cycle control can modulate apoptosis; amongst them cytokines, oncogenes,

tumour suppressor genes and cell cycle components [5,7]. From the evidence discussed above β GBP emerges as an important factor in the process which determines the shift from cell proliferation to cell death as it does not violate the apoptotic threshold of normal cells and therefore opens an optimistic window of therapeutic opportunity.

One further note of optimism is the possibility of cooperation between β GBP and apoptosis inducing drugs whose toxicity and drug resistance often hamper their therapeutic worth. Such an approach can be of particular value for drugs such as adriamycin, cisplatin and etoposide which induce apoptosis by interacting with DNA as the arrest of proliferating cells by β GBP at S/G₂ transition is characterised by a less than 4 nDNA content. One can thus envisage the successful use of apoptotic drugs at a dose both efficient in inducing apoptosis and distant from toxic levels.

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