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Biochemical Pharmacology 66 (2003) 1563–1569
Biochemical Pharmacology
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# Turning cell cycle controller genes into cancer drugs A role for an antiproliferative cytokine (βGBP)

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Received 28 February 2003; accepted 7 May 2003

**Abstract**

Cancer therapies based on drugs designed to interfere with specific targets within the molecular circuitry of cancer cells are currently under intense experimentation. Our strategy is based on the use of a naturally occurring immunomolecule which can selectively kill cancer cells, based on its ability to exploit genetic differences between normal and cancer cells. The βGBP cytokine has previously been shown to negatively regulate the cell cycle by blocking cells in late S phase. In tumour cells, but not in normal cells, the S phase block has been shown to be followed by apoptosis. Mechanisms involved in S phase arrest have been pinpointed to downregulation of signalling and altered expression of cell cycle controller proteins, including E2F1, a transcription factor with ability to play a part in apoptosis. Here we discuss the use of βGBP within the context of cancer surveillance and cancer therapeutics focussing on E2F1 as one mechanistic aspect relevant to βGBP's selective induction of programmed cell death in cancer.


*Keywords:* Cancer therapy; Drug resistance; Apoptosis; β galactoside binding protein; βGBP

## 1. Introduction

Cancer therapies are aimed at inducing apoptosis. Ideally, anticancer drugs should specifically target only neoplastic cells. They should decrease tumour burden with minimal collateral damage. In reality, the effectiveness of current conventional therapies are hampered by lack of specificity, systemic toxicity, rapid drug metabolism and both intrinsic and acquired drug resistance, multidrug resistance in particular ([1] and references within). Thus, there are occasions when chemotherapy may yield little benefit while causing collateral damage, which, when extended to progenitor cells and cells of the immune system has particular relevance. Furthermore, it cannot be excluded that cells which survive the drug challenge will not undergo genetic mutations when the drug is a genotoxic drug.

Great efforts are currently directed at the identification in cancer cells of molecular alterations which may serve as probable therapeutic targets for specially designed drugs [2–11]. Unfortunately, the undoubtedly remarkable advances in the knowledge of the molecular make-up of cancer cells has not been paralleled by an equally remarkable success by targeted therapeutics, other than when the primary event in cancer is a single targetable lesion as in the case of the $p210^{BCR\text{-}ABL}$ chimeric protein in chronic myeloid leukaemia (CML) [12–15]. The tyrosine kinase inhibitor imatinib mesylate (Glivec™) directly blocks the abl kinase activity of $p210^{BCR\text{-}ABL}$ [16], thus inhibiting CML cell growth and inducing apoptosis [17,18]. However, resistance to imatinib mesylate has been reported [19]. One main problem in major cancers (breast, prostate, colon, lung) is the multiplicity of genetic changes which by different routes promote growth, protect cells from

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*Abbreviations:* βGBP, β galactoside binding protein; CML, chronic myeloid leukaemia; PDGF, platelet-derived growth factor; RTK, receptor tyrosine kinase; MAPK, mitogen-activated protein kinase; PKB, protein kinase B; E2F, E2 promoter binding factor; IAP, inhibitor of apoptosis protein; SCID, severe combined immunodeficiency.

0006-2952/$ – see front matter © 2003 Published by Elsevier Inc.
doi:10.1016/S0006-2952(03)00512-4

apoptotic death and favour metastasis ([20] and references within). Such a multitude of changes may not always bode well for the success of therapies which are directed at single targets and whose number may be larger than those already identified. On the other hand, insight offered by Nature suggests that in the healthy organism cancer cells can be selectively eliminated. This reasoning stems from mutation probability. Theoretical molecular analysis points to $\sim 1.3 \times 10^{14}$ to $1.3 \times 10^{16}$ mutations in the protein coding region of genes in the human body, i.e. one mutation on a gene in every 7.5–750 cells. This calculation is based on a genome size of $3 \times 10^9$ bp, $3 \times 10^4$ genes, an average gene size of 2.2 kb, $10^{17}$ cell divisions to produce $10^{14}$ cells (average body mass) and a replication error rate of $10^{-11}$ to $10^{-9}$ per base. Although not all mutations will be relevant to cancer, it is a reasonable assumption that one sees fewer cancers than one might expect from these calculations. Significantly, selective elimination of cancer cells in the organism must be based on naturally occurring molecules with ability to exploit the differential dependence of normal and cancer cells on mechanisms which control mitogen activation and programmed cell death. One molecule which has been shown to have these properties is β galactoside binding protein (βGBP) a 15 kDa protein [21,22] whose gene maps in the SIS/PDGF homology region on murine chromosome 15E/human chromosome 22q12–q13 [23,24], a syntenic group found to undergo deletions and translocations in a number of human tumours [25–28]. Secreted by $CD4^+$ and $CD8^+$ activated T cells [29], but also by somatic cells [21], βGBP binds with high affinity ($K_d$ $10^{-10}$ M) to $\sim 5 \times 10^4$ receptor sites per cell to negatively control the cell cycle by down-regulating tyrosine kinase receptor-activated signalling and affecting cell cycle controller genes to induce a cell cycle pause before cell commitment to mitosis [21,30]. Remarkably, addition of the recombinant protein at nM concentrations programmes cancer cells, but not normal cells, into apoptosis [31–34]. Which molecular circuitries involved in regulating the survival/cell death equation are differentially expressed in normal and cancer cells and are specifically exploited to selectively activate programmed cell death in cancer cells is the subject of our investigations.

## 2. The cell cycle as a therapeutic target

Cancer is a disease characterised by deregulated cell proliferation, where increased mitogenic inputs require compensatory suppression of pro-apoptotic processes in order to allow uncontrolled expansion [11,35–42]. Exploiting the differential dependence on apoptosis between cancer and normal cells creates therapeutic opportunities for the selective elimination of cancers. Most currently used chemotherapeutic drugs interfere crudely with vital processes rather than targeting specific lesions within the cell cycle which are responsible for deregulated growth. Drugs designed to specifically affect identified targets include RTK inhibitors, inhibitors of members of the MAPK pathway, inhibitors of AKT/PKB ([10] and references within). Other drugs aim at re-establishing loss of function such as in the case of defective p53 or MDM2 suppression of p53 activity [43,44]. The problems facing targeted therapies concern which optimal agent to match to which lesion, the multiplicity of the lesions within the cell, and toxicity. By contrast, a natural molecule with ability to selectively eliminate cancer cells will be able to opportunistically exploit alternative routes to apoptosis created by the differential response of cancer cells to the enforcement of the molecule's function within a physiological context. This is the case of βGBP whose physiological function in normal cells is to negatively control the cell cycle by operating a specific S phase checkpoint which imposes a cell cycle pause before commitment to mitosis [21]. This is an important aspect as lack of checkpoint control may facilitate survival and carcinogenesis [45–47]. The molecular events involved in S phase checkpoint activation by βGBP include downregulation of mitogenic signalling, downregulation of cyclin A and E2F1 gene expression, decreased cyclin kinase activity and deregulation of the E2F1 transcription factor ([30] and unpublished work). Importantly, ectopic transactivation by deregulated E2F1 is a condition which leads to apoptosis where E2F1 is expressed at high levels [48–55], as can be the case in cancer cells. E2F1 can promote apoptosis through p53-dependent and -independent pathways [56,57] (Fig. 1) and force caspase enzymes to levels that may increase the probability that death inducing signals overcome inhibitors of apoptosis, such as IAP proteins [58], to impede the activation of programmed cell death. Thus, within this context, the specificity of the βGBP cytokine as a selective anticancer agent would reside not in its ability to recognize cancer cells, but in its ability to induce cell cycle regulatory changes to which normal and cancer cells respond differently.

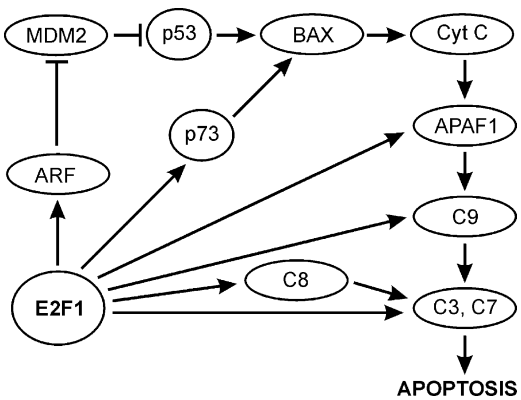



Fig. 1. Pathways involved in E2F1-induced apoptosis. These pathways are a composite from different publications.

## 3. Correlation between E2F1 and apoptosis in breast cancer

We have previously reported that βGBP arrests growth in late S phase and induces apoptosis in mammary cancer cells which differ for oncogenic potential, oestrogen receptor expression and expression of the EGF receptor family [34]. Also we have reported that in contrast to these cells, in normal luminal cells obtained from cosmetic reduction mammoplasties in short-term culture, growth arrest by βGBP is not followed by apoptosis, instead growth is resumed [34]. Here we have related the ability of βGBP to selectively induce programmed cell death in a panel of mammary cancer cells to endogenous levels of the E2F1 transcription factor. The potential importance of E2F1 in the mediation of programmed cell death in mammary cancer is indicated by the finding that E2F1 block by dominant negative mutants in MBL100 breast cancer cells inhibits apoptosis and induces tumour growth in SCID mice [59]. For this study, we have chosen MCF7 mammary cancer cells in their naïve, nascent form; multidrug-resistant MCF7/D40 and Tamoxifen-resistant MCF7/TX9 cells. As normal behaving cell counterparts, we have chosen immortalised MCF10a ductal cells, which form recognizable epithelial mammary gland pattern when in culture. The rationale for this choice was to analyse cells that, while differing for oncogenicity and drug resistance, share a common genetic denominator. Figure 2A and B shows that the predicted correlation between E2F1 levels and apoptosis held true. Apoptosis, as evidenced by the development of a subdiploid cell population (arrow), a result of progressive DNA degradation and shift of the apoptotic

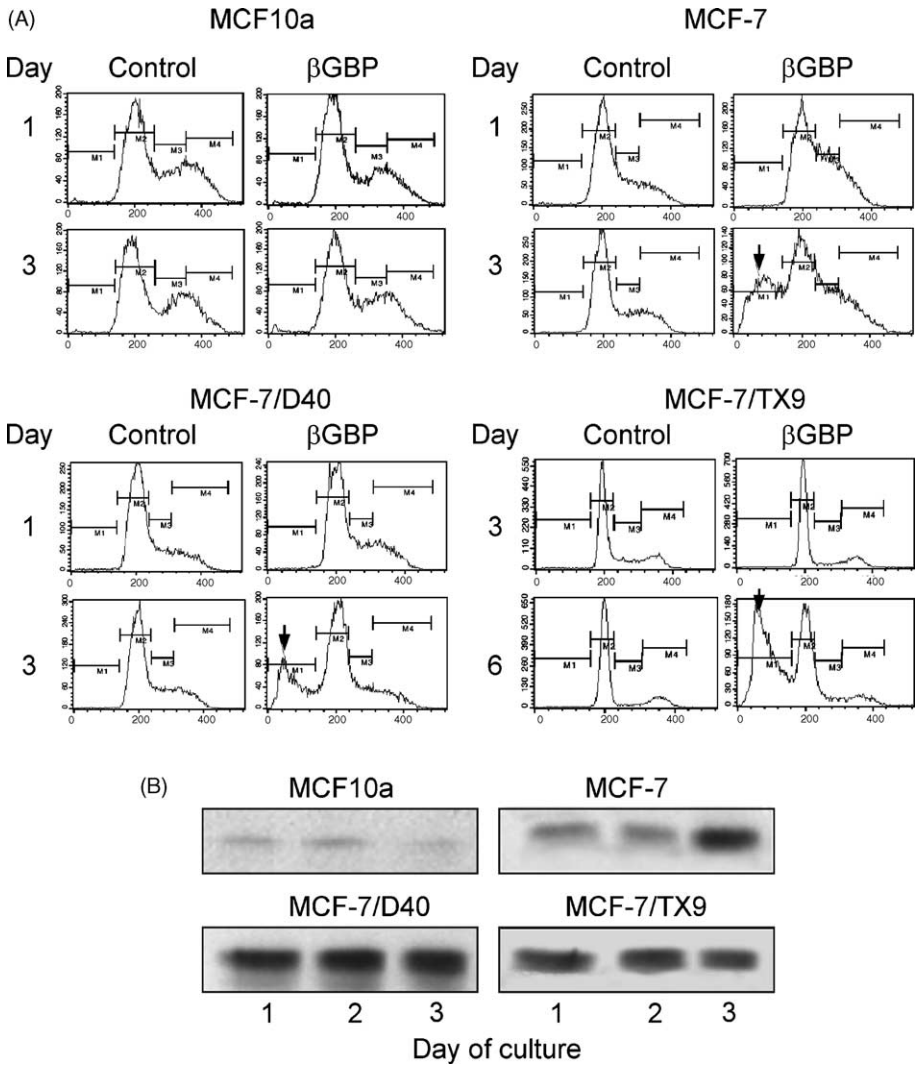



Fig. 2. Cell population and E2F1 expression in normal breast cells and in breast cancer cells. (A) Cell population assessed according to DNA content using FACS analysis. Cell arrest in S phase at day 1 is followed by apoptosis in cancer cells as indicated by subdiploid apoptotic population (arrows). Normal breast cell line MCF10a and breast cancer cell lines MCF7 and MCF7/TX9 cells were treated with 6 nM βGBP; breast cancer MCF7/D40 cells were treated with 3 nM βGBP. Immunopurified recombinant βGBP [21] added at 3 hr after seeding. Histograms represent cell population distribution assessed by propidium iodide staining from 20,000 events using a FACS calibur (Becton Dickinson). (B) E2F1 protein levels assessed by immunoblotting. E2F1 (C-20) polyclonal antibody (Santa Cruz) 1:1000. E2F1 in cancer cells remains strongly expressed after growth arrest.

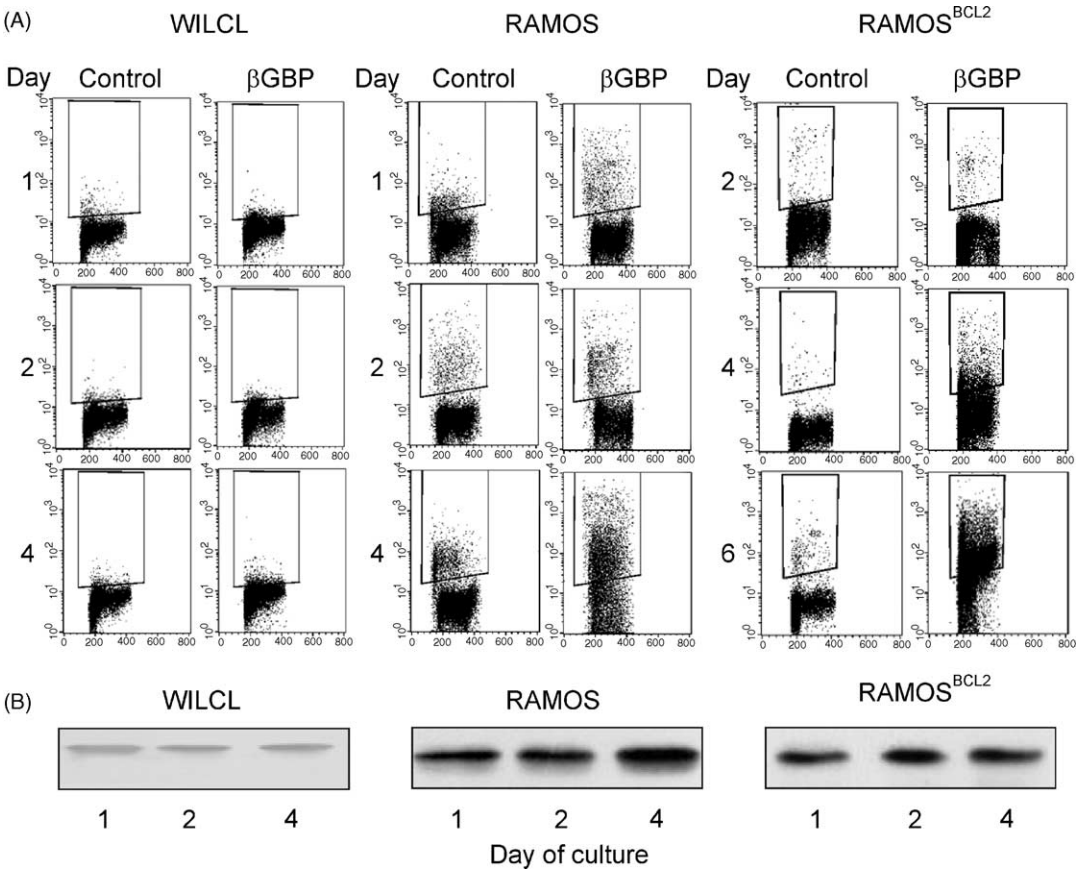



Fig. 3. Nuclear DNA fragmentation and E2F1 levels in lymphoma cells following growth arrest by βGBP. (A) Normal WILCL B cells line, p53 defective Ramos B cell lymphoma and p53 defective Ramos cells overexpressing Bcl-2, all treated with 10 nM immunopurified recombinant βGBP [21] from 3 hr after seeding. DNA fragmentation detected by end labelling of DNA strand breaks using terminal deoxynucleotide transferase [61]. Apoptotic populations in boxed areas. Cytofluorimetric analysis carried out in a FACS calibur (Becton Dickinson). (B) E2F1 protein levels assessed by immunoblotting. E2F1 (C-20) polyclonal antibody (Santa Cruz) 1:1000. E2F1 in cancer cells remains strongly expressed after growth arrest.

population into the pre-$G_1$ peak, is only evident in the three cancer cell lines where E2F1 levels are many fold higher than in the MCF10a cells where no subdiploid population is detectable.

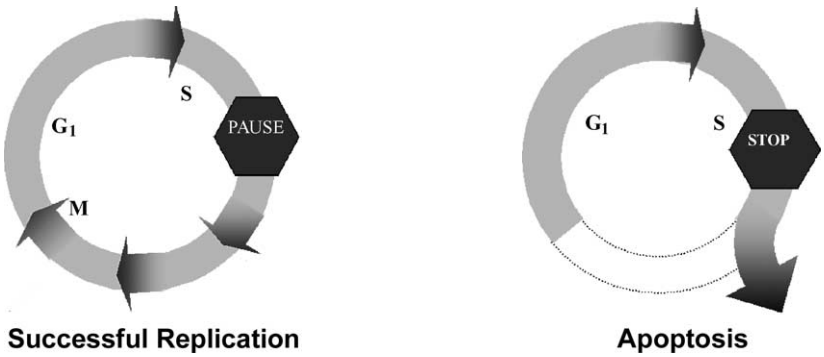
## 4. Correlation between E2F1 and apoptosis in B cell lymphoma

For this investigation, we have chosen p53 defective lymphoma Ramos cells and p53 defective Ramos cells which overexpress the Bcl-2 protein, both conditions which favour cell survival. WILCL immortalized B cells were used as normal behaving cell counterparts. Figure 3A and B demonstrates that, as in the case of the breast cancer cells (Fig. 2), apoptosis was only induced in both types of Ramos cells where E2F1 levels are strongly expressed. By contrast, the apoptotic effect of βGBP in the control WILCL cells, where E2F1 levels are many fold lower, was negligible. The evidence of Figs. 2 and 3 showing that E2F1, otherwise a short-lived protein, remains persistently expressed in cells no longer able to proliferate, indicates that E2F1 is protected from degradation and hence that it is DNA bound and able to transactivate. These conditions are in accord with the demonstrated ability of E2F1 to activate programmed cell death when E2F1 is highly expressed [52–57]. The correlation between E2F1 levels and apoptosis that we report, while offering a mechanistic understanding on means of selectively activating apoptosis in cancer cells, does not rule out that other routes which confer proliferative advantages and survival preference to cancer cells can also be affected by βGBP. We have recently found ([60] and unpublished work) that in normal cells βGBP downregulates active PI3K levels and related downstream pathways. Silencing the E2F1 gene will finally establish the extent to which the E2F1 protein is responsible for βGBP-induced apoptosis.

## 5. Effect of βGBP in normal and cancer cells. An overview

In addition to the studies reported in Sections 3 and 4 above, we report in Table 1 the effect of βGBP at concentrations ranging from 1 to 20 nM on a range of cancer cells both of epithelial and mesenchymal origin and in

Table 1
Effect of GBP on normal human cells and on human cancer cells




| Normal cells | Carcinomas | Lymphomas/leukaemias |
|---|---|---|
| Luminal breast cells | Breast | |
| From cosmetic mammoplasties. Examined in parallel with BT20 and T47D | BT20; T47D; BT474; SKBR3; MCF7 | ST4; PF382; Jurkat; Raji; JM1 |
| Expanding T cells | Tamoxifen resistant MCF7/TX9 | Daudi; Ramos $p53^{-}$; Ramos $p53^{-}$/Bcl-$2^{+}$ |
| From volunteers. Examined in parallel with ST4, PF382 and Jurkat. | Multidrug resistant MC7/D40 | |
| Progenitor cells | Colon | |
| From volunteers. Examined in clonogenic assays in parallel with cells from CML patients at diagnosis. | CCL 233; SW480; SW620; LoVo; HT29 | U937 |
| | Colchicin resistant HT29/VMDR | Topotecan resistant U937/RERC |
| | Ovarian | |
| | 2008 | CML |
| | Cisplatin resistant 2008 CP | K562; BV173 |
| | Oral | LAMA-84; KYO1 |
| | KB-3-1 | CML from patients at diagnosis |
| | Vinblastine resistant KB-V-1 | |

normal cells taken with consent from healthy donors. These latter cells are of particular significance as they demonstrate that βGBP caused them no significant harm. Instead their response to βGBP was that expected from the protein acting as a physiological negative cell cycle regulator. This is in contrast with the response of the cancer cells examined where cell cycle arrest was followed by apoptosis. Three outstanding patterns emerge from Table 1: the extensive range of cancer cells which responded to βGBP by entering programmed cell death following cell cycle arrest; the equal efficiency of βGBP on cells resistant to a variety of drugs; the lack of harmful effect on ex vivo cells, including cells of the immune system and progenitor cells.

## 6. Conclusion

From an improved understanding of mechanisms responsible for deregulated cell proliferation in cancer cells novel strategies aimed at restoring apoptosis sensitivity have been lately emerging. These strategies are based on the design of drugs intended to kill cancer cells within a molecular context which matches that of a targeted cancer cell. However, our current knowledge of the cell death machinery is still incomplete and it will be a daunting challenge to have a complete wiring diagram of structures of optimal physiological relevance to the modulation of signals and protein–protein interactions leading to apoptosis.

We have allowed the organisms to give us suggestions. Our strategy is based on the use of a small immunomolecule produced by T cells [29] which offers credentials to qualify as a cancer surveillance molecule with the ability to perform therapeutically what the protein would naturally perform within the healthy organism, and it is perhaps more than an assumption that its disappearance, forced by conditions such as immunosuppression, would increase propensity to cancer.

βGBP negatively regulates the cell cycle in normal cells [21]; in cancer cells, βGBP causes cell arrest and induces apoptosis [30–34]. We have shown one mechanistic aspect relevant to the selective activation of programmed cell death: persistent expression of high levels of E2F1 in cancer cells unable to proliferate when exposed to nanomolar concentrations of βGBP. We have also shown that a wide range of cancer cells, including drug resistant ones, succumb to βGBP, while very sensitive normal cells such as T cells and progenitor cells are not harmed by the treatment. The identification of other routes that may be exploited by βGBP to selectively induce apoptosis in cancer, further to adding to the picture of the potential

therapeutic worth of this protein, may possibly indicate new targets for drug design.

## Acknowledgments

We apologise to those whose work was not cited or discussed due both to abundance of literature and to space limitations. We thank Kate Kirwan for skilful and patient artwork and Paul Devlin for calculation of mutation probability. We also thank Mark Lawler (Trinity College, Dublin), Renato Mariani-Costantini and Lavinia Lotti (La Sapienza University, Rome) and Khew-Voon Chin (New Jersey Cancer Institute) for personal communications concerning studies on CML and on drug resistant cells. The work by L.M. and V.W. was funded by the Breast Cancer Campaign, UK.

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