Killing of Kras-Mutant Colon Cancer Cells via Rac-Independent Actin Remodeling by the βGBP Cytokine, a Physiological PI3K Inhibitor Therapeutically Effective In Vivo

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Abstract

Activating mutations in Kras are the most frequent mutations in human cancer. They define a subset of patients who do not respond to current therapies and for whom prognosis is poor. Oncogenic Kras has been shown to deregulate numerous signaling pathways of which the most intensively studied are the Ras/extracellular signal–regulated kinase cascade and the phosphoinositide 3-kinase (PI3K)/Akt cascade. However, to date, there are no effective targeted therapies in the clinic against Kras-mutant cancers. Here, we report that the β-galactoside–binding protein (βGBP) cytokine, a physiologic inhibitor of class I PI3Ks, is a potent activator of apoptosis in Kras-mutant colorectal cancer cells, even when coharboring mutant-activated PIK3CA. Our study unveils an elective route to intrinsic and extrinsic apoptosis, which involves the cytoskeleton. Early events are inhibition of PI3K activity and Rac-independent actin rearrangement assignable to phosphoinositide changes at the plasma membrane. Cyclin E deregulation, arrest of DNA synthesis, and checkpoint kinase 2 activation underscore events critical to the activation of an intrinsic apoptotic program. Clustering of CD95/Fas death receptors underscores events critical to the activation of extrinsic apoptosis. In nude mice, we present the first evidence that xenograft tumor development is strongly inhibited by Hu-r-βGBP. Taken together, our results open a new therapeutic opportunity to a subset of patients refractory to current treatments. This first demonstration of therapeutic efficacy against Kras-mutant colon cancer suggests that Hu-r-βGBP may also be therapeutically effective against other cancers harboring activating Ras mutations as well as PIK3CA mutations. Mol Cancer Ther; 11(9); 1–10. ©2012 AACR.

Introduction

Activating mutations in the Ras genes (H,K,N; ref. 1), the most common oncogenic mutations in human cancers, are dominant determinants of drug resistance. Most frequent in Kras, Kras mutations define tumors refractory to conventional chemotherapy and radiation therapy (2, 3) and to more recently introduced therapies based on EGF receptor tyrosine kinase inhibitors (4–7). Locked in the GTP-bound mode, oncogenic Ras constitutively activates the Raf/MEK/extracellular signal–regulated kinase (ERK) cascade and an integrated signaling network, which controls cell proliferation and cell survival (8, 9) to be a major mediator of tumorigenesis.

The importance of activating mutations in the Ras oncogenes in human cancers has made Ras and downstream effectors elective targets for therapeutic intervention, but the value of targeting inhibitors in the clinic remains doubtful. For example, prevention of Ras anchorage at the cell membrane by farnesyltransferase inhibitors has been shown to be effective in mice expressing oncogenic Ras but not in patients with solid tumors (10). Similarly, in more recent studies, CI-1040, a MEK inhibitor, was found to induce significant shrinkage of Kras-driven lung carcinomas in mice (11), but had no significant effect in patients with advanced lung, breast, colon, and pancreatic cancers (12). Using a different approach, a number of compounds with efficacy against cells with mutant-activated Kras have been identified by high-throughput screening (13–16) and shown to inhibit xenograft growth (13,15, 16); however, the mechanisms by which these compounds operate are not fully understood and their anticancer efficacy in the clinic remains uncertain.

Recently, the necessary role played by the phosphoinositide 3-kinase (PI3K)/Akt pathway in maintaining tumor growth has made this pathway a target for small...
molecule inhibitors of the PI3K cascade (17–21). However, the efficacy of these inhibitors as single agents in Kras-driven oncogenesis is disputable. For example, in mice that had developed lung tumors in response to Kras activation, no significant tumor regression was found upon treatment with a dual pan-PI3K/mTOR inhibitor, which had instead shown efficacy in lung tumors driven by the expression of activated PI3K (22). In other experiments, the efficacy of the PI3K inhibitor PX-866 was significantly less in xenografts bearing Kras and PIK3CA mutations than in those having PIK3CA mutations alone (23). On the other hand, Kras-mutant lung tumors did regress when the pan-PI3K/mTOR inhibitor was used in combination with an MEK inhibitor (22). Similarly, in cells expressing Kras or Hras mutants, resistance to PI3K inhibitors could be reversed by inhibitors of the Ras/ERK pathway (24). Despite these positive results, it remains to be established whether in the human system Kras oncogenicity could be overcome by the combined targeting of the PI3K and the Ras pathways and be beneficial as, in addition to toxicity, a disadvantage of this approach is that these pathways are also required for the proliferation of normal cells and for the maintenance of their homeostatic balance.

In previous studies, we have shown that monomeric \( \beta \)-galactoside–binding protein (\( \beta \)GBP), an antiproliferative cytokine (25) that in normal cells participates in the negative regulation of the cell cycle (26), operates through mechanisms that involve high-affinity receptor binding (\( K_d \approx 1.5 \times 10^{-10} \) mol/L; ref. 26) and molecular interactions leading to downregulation of class IA and class IB PI3Ks (27). Inhibition of PI3K activity by Hu-r-\( \beta \)GBP was found to have two major outcomes: suppression of Ras-GTP loading, leading to a block of ERK activation (27) and negation of Akt gene expression leading to loss of Akt (21), conditions that either by blocking the ability of cancer cells to proliferate or by impairing their ability to survive can block oncogenicity, thus highlighting a selective anticancer effect as in normal cells, \( \beta \)GBP-enforced cell cycle restriction is reversible (26).

We now report that in colorectal carcinoma cells bearing oncogenic Kras downregulation of PI3K activity by Hu-r-\( \beta \)GBP at doses that did not inhibit canonical signaling downstream of Ras or PI3K promoted events critical to the activation of an intrinsic and an extrinsic cell death program initiating with Rac-independent actin reorganization assignable to phosphoinositide changes at the plasma membrane. We also report that Hu-r-\( \beta \)GBP had therapeutic efficacy in vivo and that the added presence of activating mutations in PIK3CA did not confer resistance to treatment with Hu-r-\( \beta \)GBP.

### Materials and Methods

#### Cells

SW480, SW620, and LoVo cells were acquired from the American Type Culture Collection and cultured in
Leibovitz's L-15 medium with 10% fetal bovine serum. HCT116wt, HCT116mut, DLD1wt, and DLD1mut cells, a gift from Bert Vogelstein (John Hopkins University, Baltimore, MD), were cultured as originally reported (28). All cell lines were authenticated. Cell population distribution was assessed by propidium iodide staining and fluorescence-activated cell-sorting analysis as reported previously (26). Epithelial cells of the normal colon mucosa from surgical interventions were obtained with informed consent and the study was approved by the Medical School's Ethics Committee.

Recombinant βGBP
Hu-r-βGBP was expressed in Escherichia coli BL21 (DE3) using hGal-1 cDNA in PET21a, purified by lactose–agarose (Sigma) affinity chromatography and purity assessed by matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF).

Apoptosis assays
Tetramethylrhodamine ethyl ester (Molecular Probes/Invitrogen) staining, Annexin V (Pharimingen) staining, and caspase-3 activity (Oncommunnin) were assessed...
PI3K assay
Step-by-step description of the method for assessment of PI3K activity has been reported previously (21). In brief, the immunoprecipitated class I enzyme complex (27) was incubated in a kinase reaction for 3 hours with 40 pmol phosphatidylinositol (4,5)-biphosphate (PIP2) substrate and the phosphatidylinositol (3,4,5)-triphosphate (PIP3) generated assayed in a competitive ELISA (Echelon Biosciences). Differences were tested using Student t test and P < 0.05 was considered statistically significant.

Western blotting
ERK2 phosphorylation was visualized by mobility shift using anti-p42 polyclonal antibodies (Santa Cruz Biotechnology). Phosphorylated Akt was detected by antiphospho-Akt (Ser473) antibody (Cell Signaling Technology) and total Akt1/2 protein probed with anti-Akt1/2 antibodies (Santa Cruz Biotechnology). E2F1 was detected using anti-E2F1 polyclonal antibodies (Santa Cruz Biotechnology) and phosphorylated checkpoint kinase (Chk) 2 detected with antiphospho-Chk2 (Thr 68) polyclonal antibodies (Cell Signaling Technology). Secondary antibodies conjugated to horseradish peroxidase (GE Healthcare) were used for visualization by enhanced chemiluminescence (GE Healthcare). Blots were reprobed with anti-glyceraldehyde-3-phosphate dehydrogenase (Santa Cruz Biotechnology) or with monoclonal anti-β-actin (Sigma). Active Rac1 levels were assessed by affinity precipitation using PAK1 p21 binding domain agarose (Millipore) according to the manufacturer’s instructions, followed by immunoblot analysis using anti-Rac1 monoclonal antibody (Cell Signaling) and Odyssey fluorescent secondary antibodies. Total Rac was assessed using the same antibodies.

Fluorimetric quantitation of cyclin E
Cells fixed in cold 70% ethanol were treated with fluorescein isothiocyanate (FITC)-labeled monoclonal anti-cyclin E antibody (Becton Dickinson), washed, stained with propidium iodide containing RNase (Sigma), and analyzed using an LSRII (Becton Dickinson). FITC fluorescence was collected using a 530/30 filter and propidium iodide fluorescence using a 610/20 filter. At least 20,000 events were recorded.

DNA synthesis
Cells incubated with bromodeoxyuridine (BrdU; Sigma) for 30 minutes were fixed in cold 70% ethanol and stained for BrdUrd uptake using a monoclonal anti-BrdU antibody (Becton Dickinson), followed by an FITC-labeled monoclonal goat anti-mouse antibody (Dako). DNA was counterstained with propidium iodide (Sigma) containing RNase (Sigma). Samples were analyzed using an LSRII (Becton Dickinson) and FITC fluorescence collected using a 530/30 filter and propidium iodide fluorescence using a 610/20 filter. At least 20,000 events were recorded.

Microscopy
Cells on coverslips fixed in 3% paraformaldehyde/0.2% gluteraldehyde solution were stained with Texas Red phalloidin (Molecular Probes) for F-actin detection. For tubulin detection, cells were treated with a monoclonal anti-α-tubulin antibody (Sigma) followed by anti—mouse secondary antibody labeled with Alexa Fluor 488 (Molecular Probes). DNA was stained with 4’, 6-diamidino-2-phenylindole (DAPI, Sigma). Imaging was carried out using a Zeiss LSM 510 confocal system. Analysis of the F-actin–labeled regions was conducted in an automated manner using journals in MetaMorph software (Universal Imaging) and statistical significance tested by ANOVA (29). For CD95/Fas and FasL imaging with a Zeiss Axiovert 200M and a Zeiss Axiophot microscope, cells were fixed in 4% paraformaldehyde, incubated with a monoclonal antibody to CD95/Fas (Abcam) followed by FITC-conjugated goat anti-mouse immunoglobulin G (IgG; Cappel) and rabbit polyclonal FasL antibody (Santa Cruz Biotechnology), followed by Texas red–conjugated goat anti-rabbit IgG (Jackson Immunoresearch Laboratories). Time-lapse imaging by differential interference contrast microscopy was used to monitor peripheral dynamics at the plasma membrane edge. Recordings on sets of 150 cells from 3 randomly selected fields from double experiments were analyzed and graded on a 0 to 3 arbitrary scale by 2 independent observers and statistical significance determined by χ² test.

| Table 1. Quantitation of cell spread area as defined by F-actin staining |
|---------------------------|---------------------------|---------------------------|---------------------------|
|                         | F-Actin spread area at 24 h, μm² | F-Actin spread area at 48 h, μm² |
|                         | Control | βBGP | P     | Control | βBGP | P     |
| SW480                   | 187.2 ± 4.0 (278) | 216.7 ± 4.9 (229) | <0.01 | 192.3 ± 3.6 (414) | 236.6 ± 9.0 (117) | <0.001 |
| SW620                   | 190.7 ± 3.4 (330) | 219.3 ± 4.9 (208) | <0.01 | 212.8 ± 4.3 (289) | 245.1 ± 5.8 (219) | <0.01 |
| LoVo                    | 183.4 ± 4.0 (221) | 224.8 ± 4.0 (342) | <0.001| 193.1 ± 2.7 (707) | 250.4 ± 6.7 (179) | <0.001 |

NOTE: Values are means ± SEM. Data acquired using MetaMorph software and analyzed by ANOVA (29) in Mathematica 4.2. Values in brackets represent the number of cells measured.
Tumor growth in mice

Thymectomized female nude CD-1 mice (Charles River Laboratory) were implanted subcutaneously in the right flank with $5 \times 10^6$ SW620 cells in 150 μL PBS. Control mice received an equal volume of PBS. Mice were ear marked and randomized in groups of 7 and treatment with Hu-βGBP dissolved in PBS started at a tumor volume of approximately 40 mm$^3$. Treatment was carried out by
s.c. injection of 150 µL in the tumor area for 6 consecutive days with 1 day interval, up to 35 days. Control mice received an equal volume of PBS. Experiments were carried out in accordance with the U.K. Coordinating Committee on Cancer Research guidelines and approved by the local responsible authorities.

**Results**

**Arrest of cell replication and apoptosis is preceded by cytoskeletal rearrangement**

We tested Hu-r-βGBP against SW480 and SW620 colorectal cancer cells, of primary and metastatic derivation, both harboring KrasG12V, and against LoVo cells, also of metastatic origin, which harbor KrasG13D (COSMIC database). We found that treatment with Hu-r-βGBP at concentrations of 2–4 nmol/L, some 10-fold lower than those required for other cancer cells (21, 30), resulted in growth arrest and accumulation of cells in S-phase, a state reversible in normal cells (26) but followed in the cells of this study by total cell loss attributable to the activation of an apoptotic program documented by changes in mitochondrial membrane potential, functional alteration of the plasma membrane, and caspase-3 activation (Fig. 1A and B).

We next investigated whether as in other cell contexts (21, 27) PI3K was a responder to the action of Hu-r-βGBP. As cell phosphoinositide levels do not directly represent the functional state of the PI3K enzymes, but are the result of PI3K and PTEN activity, to estimate PI3K activity we isolated the PI3K enzyme complex (27) by immunoprecipitation and assessed its ability to convert PIP2 into PIP3 in a kinase reaction by measuring the generated PIP3 in a competitive ELISA (21). PIP3 quantitation (Fig. 2A) shows that downregulation of PI3K activity was a prime event, although delayed in the LoVo cells. However, contrary to previous evidence from other cell types (21, 27), we found no indication, after PI3K inhibition, of loss of ERK or Akt function in which phosphorylation state in the cells programmed to death was instead increased (Fig. 2B), a response conceivably similar to that seen in human cancer cells in which inhibition of mTORC1 by a rapamycin derivative resulted in increase ERK and Akt activity via a feedback loop (31). We discovered instead that the treated cells had undergone a profound change of shape characterized by F-actin rearrangement, spreading of the microtubular network, and an enlargement of the cell spread area in which changes in time were quantitated in terms of F-actin spatial distribution (Fig. 2C and Table 1), changes that can block cancer cell motility and invasion (32) but in our case followed by apoptosis (Fig. 1B).

To investigate whether actin remodeling related to changes in Rac activity, a key regulator of actin dynamics, we assessed active Rac1 levels by immunoblot analysis and examined protrusive activity at the membrane edge, a Rac-mediated process (33), by time-lapse imaging within the 24 hours time span, leading to cytoskeletal changes. We found that in the cells in which PI3K activity had been inhibited, Rac1 levels remained unchanged and edge membrane activity was not affected by treatment with Hu-r-βGBP or by treatment with wortmannin (10 µM), a pharmacologic p110 inhibitor. These results, consistent with the finding that edge ruffling induced by constitutively active Rac is not blocked upon PI3K inhibition (34), suggest that in cells harboring oncogenic Ras, Rac activation is maintained through Ras-mediated signaling in a PI3K independent manner (35).

**Cyclin E stabilization and arrest of DNA synthesis result in Chk2 activation**

To determine which biochemical events consequent to PI3K inhibition and cytoskeletal changes may play a part

![Figure 4. Localization of CD95/Fas and FasL in controls and in treated cells. Control cells show punctate staining for both CD95/Fas (green) and FasL (red) with low levels of colocalization (bottom, merge). Treated cells show clustering of CD95/Fas (green) and clustering of FasL (red), which can be frequently found at the same localization on cell surfaces (bottom, merge). Examples of this clustering are indicated by arrowheads. Hu-r-βGBP 4 nmol/L was added 6 hours after seeding. SW480 cells fixed at hour 24 of treatment, SW620 and LoVo cells fixed at hour 48. Scale bars, 10 µm.](#)
in cell-cycle arrest and in the activation of an apoptotic program, we examined parameters involved in cell growth restriction.

Following immunoblot analysis, which in the arrested cells revealed no changes in cyclin D1 levels but raised and persistent levels of cyclin E and a gradual, and expected, decline of cyclin A, we quantitated cyclin E by cytofluorometry and found that while decreasing in the replicating cells, cyclin E levels in the arrested cells remained ectopically high, with resulting relative values greater than those of controls by about 60% to 90% (Fig. 3A). As ectopic expression of cyclin E can cause impairment of DNA replication due to defects in replication initiation (36), we investigated whether in the arrested cells DNA synthesis had been inhibited. We found that by day 2 of treatment, DNA synthesis had come to a halt (Fig. 3B). Because processes that interfere with DNA synthesis may result in the activation of DNA damage checkpoints and death by apoptosis (37), we turned our attention to the downstream kinase effectors of the DNA damage response pathways (37–39). Chk2 was not detectable but we found that Chk2, which is required for responses to DNA damage and replication block (37, 39), had been activated (Fig. 3C). This is of relevance as Chk2 can phosphorylate E2F-1, regulating its stability and transcriptional activity (38, 40), and be a cause of apoptotic induction in cells in which, in contrast to normal cells, E2F-1 is overexpressed (41, 42), as can be seen when comparing epithelial cells from the normal colon mucosa and the colon cancer cells of this investigation (Fig. 3D and E).

**Involvement of death receptors**

Further to the rearrangement of cytoskeletal architecture (Fig. 2B), it is conceivable that rearrangement in the organization of subcortical actin may affect macromolecular mobility within the plane of the plasma membrane. We therefore investigated whether treatment with Hu-r-βGBP would affect the distribution pattern of the CD95/Fas death receptor which, as well as other death receptors (28), is a mediator of apoptosis, and examined whether the distribution pattern of Fas L would change accordingly. The evidence collected (Fig. 4) shows that as suggested by their colocation, CD95/Fas death receptor which, as well as other death receptors, is a cause of apoptotic induction in cells in which, in contrast to normal cells, E2F-1 is overexpressed (41, 42), as can be seen when comparing epithelial cells from the normal colon mucosa and the colon cancer cells of this investigation (Fig. 3D and E).

**Hu-r-βGBP has efficacy in vivo**

Next, we tested whether the therapeutic efficacy observed in vitro could be reproduced in an in vivo model. We implanted subcutaneously SW620 metastatic cells in nude mice and initiated treatment at a tumor volume of approximately 40 mm³ administering Hu-r-βGBP at doses conforming to the nanomolar in vitro dosages (Fig. 5) used in the current and in previous in vitro experiments (21, 27, 30). Comparison of growth curves (Fig. 5A) shows that a 5-week period of treatment had inhibited xenograft growth by approximately 70%, with none of the treated mice appearing unhealthy as assessed by observation of behavior and body weight measurements.

On further observation once the treatment had been stopped, we followed individually surviving mice bearing small tumors (~100–200 mm³; Fig. 5B) and compared the rate of tumor growth resumption with the
growth rate of control xenografts from an average volume of approximately 100 mm$^3$ hence. Histograms show that resumption of tumor development proceeded at a slower rate and that in only 2 instances within a 9-week period xenograft size had approached the size attained in 3 weeks by the xenografts of the control mice (Fig. 5C).

**Mutant PIK3CA does not confer resistance to Hu-r-βGBP**

In addition to oncogenic Kras, in which frequency in human colorectal cancer is about 40%, activating mutations in the PIK3CA gene, also frequent in colorectal cancer (43), can induce oncogenicity (44) and tumor invasion (45). To investigate whether Hu-r-βGBP would be therapeutically effective in colorectal cancer cells bearing both mutations, we examined HCT116 and DLD1, both mutated at Kras-G13D (13) in which either the mutant or the wild-type PIK3CA allele had been deleted by homologous recombination to express 1 of 2 major hot spot mutations (44). In a tightly controlled experiment, we examined HCT116$^{[G13D]}$ cells harboring an H1047R alteration in exon 20 (kinase domain) and DLD1$^{[G13D]}$ cells, which have an E545K alteration in exon 9 (helical domain) paired with their isogenic wild-type counterparts. Our results show that compared with the wild type, the growth rate of cells harboring mutant PIK3CA was increased and apoptosis was delayed by 1 day (DLD1mut; Fig. 6A and B), but the response to Hu-r-βGBP was fundamentally similar in all cells whether PIK3CA wild type or mutant and similar to the response of the colorectal carcinoma cells of Figure 1, which are exempt of PIK3CA mutations. Inhibition of PI3K activity was followed by inhibition of cell proliferation and cell death assignable to the activation of an apoptotic program.

**Discussion**

Mutant-activated Kras defines a subset of patients with poor prognosis. We show that the PI3K inhibitor used in this study was remarkably efficacious. Hu-r-βGBP as a single agent induced apoptotic death in cancer cells harboring mutant-activated Kras, even when coexpressing an activating mutation in PIK3CA, and was therapeutically effective in vivo.

Unlike molecules designed to target critical hubs within signaling pathways, the βGBP molecule operates through mechanisms, which involve high-affinity receptor binding (26) and molecular interactions leading to functional inhibition of the p110 catalytic subunit of class IA and class IB PI3Ks with consequent loss of ERK (27) and Akt function (21). Surprisingly, in the cancer cells of this study, inhibition of PI3K activity was not characterized by loss of active ERK or active Akt, key prognostic therapeutic markers to βGBP response (46), whose phosphorylation state was instead increased but characterized by actin reorganization in a Rac-independent manner.

Although our data provide no indication that changes in actin organization were related to loss of Rac activity, the fact that phosphoinositides and PI2P in particular have an important role in the binding and control of actin regulatory proteins (47) lends ground to infer that changes in PI2P levels, turnover, or spatial distribution brought about by molecular interactions involved in βGBP receptor–PI3K communication (27) may affect plasma membrane–cytoskeletal linkages and have a causative role in the activation of 2 distinct sets of events critical to the

![Figure 6](https://example.com/figure6.png)  
**Figure 6** Hu-r-βGBP overcomes Kras and PIK3CA mutations. A, growth curves show rate of cell proliferation as related to dose response in PIK3CA wild-type (wt) and in PIK3CA mutant (mut) colorectal cancer cells. Values are means of triplicate cultures ± SEM. Inset histograms represent PI3K activity as measurements of PI3P expressed as percentages of controls. Values are means of triplicate measurements ± SEM. *, P < 0.05 versus controls. Hu-r-βGBP 8 nmol/L or 12 nmol/L (DLD1mut) was added 6 hours after seeding. B, loss of mitochondrial membrane potential assessed by tetramethylrhodamine ester (TMRE) staining at day 4 or 5 (DLD1mut). Inset values are percentages of cells committed to apoptosis (encircled area). Hu-r-βGBP was added 6 hours after seeding.
initiation of an apoptotic program. One relating to a
general rearrangement of the cytoskeleton, consequent
cell-cycle checkpoint restrictions, and the ectopic accu-
mulation of cyclin E in which bearing on the impairment of
DNA replication provides a potential for errors in DNA
repair and for the activation of an intrinsic apoptotic
process. The other set of events relates, conceivably, to
changes in cortical actin organization, increased macro-
molecular mobility within the plane of the plasma mem-
brane, and clustering of death receptors, a critical condi-
tion for the activation of an extrinsic apoptotic process.

Two other aspects of our study deserve mention. One
to the importance that activating mutations in the
PIK3CA gene have in conferring advantages that facilitate
cell growth and invasion (44). Our data (Fig. 6) provide
strong evidence that in colorectal carcinoma cells harboring
PIK3CA further to oncogenic Ras, inhibition of PI3K activity
was followed by the activation of a cell death program, underscoring that as single agent, Hu-r-βGBP had full therapeutic efficacy where combination targeting
of PI3K and of Ras downstream effectors may instead
be required (22, 24). The other key aspect relates to the
ability of Hu-r-βGBP to strongly reduce tumor growth and
to convert tumors that had survived a 5-week period
of treatment from a faster to a slow developing (Fig. 5B
and C) and, conceivably, a more benign tumor phenotype.

On the basis of a model discussed in a previous report (21),
this result may be interpreted as proof-of-principle evi-
dence that cancer vulnerability in response to Hu-r-βGBP challenge is greater in the aggressive cancer phenotype, as
a contained tumor growth after the end of treatment is
suggestion of clonal selection through elimination of the
more aggressive cells.

Recently, a number of compounds identified by high-
throughput screening have been reported to have therapeu-
tic efficacy against Kras-mutant cancer cells (13–16); however, among all candidate agents for the therapy of
cancers harboring mutant-activated Ras, the βGBP mole-
cule, a cytokine, by virtue of its physiologic nature is the
only one, to our knowledge, which in the clinic would be
implicitly exempt from drug toxicity and drug resistance.
As, further to colorectal carcinoma, mutations in the Ras
oncogenes and in genes involved in PI3K regulation
extend to other cancers, our results suggest that Hu-r-
βGBP is, potentially, a therapeutic agent that could pro-
vide benefit to a large number of patients.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: L. Mallucci, D.-y. Shi,
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Administrative, technical, or material support (i.e., reporting or organ-
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Study supervision: L. Mallucci, V. Wells, D. Zicha

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