Putative role for the GTPase, hGBP-1, in tumor cell proliferation and resistance to paclitaxel

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Putative Role for the GTPase, hGBP-1, in Tumor Cell Proliferation and Resistance to Paclitaxel

By

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Cell proliferation and cell death are essential, yet opposing, cellular processes that maintain homeostasis. Disruption of these programmed cellular processes may lead to cancer progression, often accompanied by resistance to chemotherapy. During cancer progression, interferons production can lead to the induction of human Guanylate-Binding Protein-1 (hGBP-1). hGBP-1 retards the proliferation of endothelial cells but its role in carcinomas is not fully understood. The present study demonstrates that ectopic expression of hGBP-1 inhibits the proliferation of MCF-7 breast cancer and 2fTGH sarcoma cells, but its expression in DU145 prostate cancer cells does not influence their proliferation. In contrast, IFN-γ or IFN-2αA treatment does not influence the proliferation of MCF-7 and 2fTGH cells, but the proliferation of DU145 cells is inhibited by IFN-α2a treatment. This indicates that interferons do not universally inhibit the proliferation of carcinomas. Some breast and ovarian cancer cell lines show elevated expression of hGBP-1 when they are made resistant to paclitaxel. The ectopic expression of hGBP-1 in OVCAR8 cells protects the cells from paclitaxel-induced cytotoxicity. This protection by hGBP-1 is also provided in MCF-7 breast cancer and
SKOV-3 ovarian cancer cells, through inhibition of paclitaxel-induced apoptosis. The expression of hGBP-1 is not always elevated in paclitaxel-resistant MCF-7 breast and A2780 ovarian cancer cell lines suggesting that up-regulation of hGBP-1 is not the only mechanism for the development of resistance to paclitaxel. Analysis of the levels of hGBP-1 mRNA in primary and recurrent ovarian tumors demonstrates that up-regulation of the expression of hGBP-1 is associated with the development of paclitaxel or docetaxel resistance in ovarian cancer patients. Elevated expression of TUBB3, a β-tubulin isotype, can also be involved in paclitaxel-resistance in carcinoma cells. In ovarian tumors, the level of TUBB3 mRNA is elevated prior to chemotherapy, suggesting that elevated expression of TUBB3 may be associated with intrinsic resistance to paclitaxel treatment. Moreover, the expression of TUBB3 is not strictly associated with the expression of hGBP-1 in primary and recurrent tumors. Together, these results suggest that hGBP-1 may be a diagnostic marker for the development of resistance to taxane treatment in ovarian tumors.
I dedicate my dissertation work to my Ma and Papa. A special thanks to my loving parents, Sipra and Sudip Bose. Their words of encouragement and push for tenacity ring in my ears which gave strength to work. I would also like to thank my dearest uncle, Sushant Bose for his constant inspiration.
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<td>AKT</td>
<td>Protein kinase B</td>
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<tr>
<td>AIF</td>
<td>Aluminum fluoride</td>
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<td>ATG</td>
<td>Autophagy related proteins</td>
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<td>ATP</td>
<td>Adenosine triphosphate</td>
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<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>bp</td>
<td>Base pairs</td>
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<td>BrDU</td>
<td>Bromodeoxyuridine</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complimentary DNA</td>
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<tr>
<td>cRNA</td>
<td>Complementary RNA</td>
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<td>C-terminus</td>
<td>Carboxy-terminus</td>
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<td>CTP</td>
<td>Cytidine 5’-triphosphate</td>
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<td>Dimethyl Sulfoxide</td>
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<td><em>Encephalomyocarditis virus</em></td>
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<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>2fTGH</td>
<td>Human sarcoma cells</td>
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<tr>
<td>GAS</td>
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<td>Guanosine diphosphate</td>
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<tr>
<td>GMP</td>
<td>Guanosine monophosphate</td>
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<tr>
<td>GppNHp</td>
<td>5’-Guanyllyl-imidodiphosphate</td>
</tr>
<tr>
<td>GRA15</td>
<td>Dense granule protein 15</td>
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<td>Guanosine triphosphate</td>
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<td>GTPase</td>
<td>Guanosine triphosphatase</td>
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<td>HCV</td>
<td><em>Hepatitis C virus</em></td>
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<td>hGBP</td>
<td>Human Guanylate-Binding Protein</td>
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<tr>
<td>hGBP-1FL</td>
<td>Full length human Guanylate-Binding protein-1</td>
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<td>Human umbilical vein endothelial cells</td>
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<td><em>Influenza A virus</em></td>
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<td>IFN</td>
<td>Interferon</td>
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<td>Immunoglobulin G</td>
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<td>Interleukin</td>
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<td>IRF</td>
<td>Interferon regulatory factor</td>
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<td>Interferon response sequence</td>
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<td>Interferon-stimulated gene factor 3</td>
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<td>--------------</td>
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<tr>
<td>ISRE</td>
<td>Interferon-stimulated response element</td>
</tr>
<tr>
<td>LC3</td>
<td>1α/1β-light chain 3</td>
</tr>
<tr>
<td>LG</td>
<td>Large globular</td>
</tr>
<tr>
<td>Lm</td>
<td><em>Listeria monocytogenes</em></td>
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<tr>
<td>Mb</td>
<td><em>Mycobacterium bovis</em></td>
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<tr>
<td>MCF-7</td>
<td>Human breast adenocarcinoma cells</td>
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<td>MDR1</td>
<td>Multidrug resistance gene</td>
</tr>
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<td>MEVCs</td>
<td>Microvascular endothelial cells</td>
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<tr>
<td>mGBP</td>
<td>Murine Guanylate-Binding Protein</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<td>mRNA</td>
<td>messenger RNA</td>
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<tr>
<td>MTOC</td>
<td>Microtubule-organizing center</td>
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<tr>
<td>NADPH oxidase</td>
<td>Nicotinamide adenine dinucleotide phosphate oxidase</td>
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<td>NF-κB</td>
<td>Nuclear factor kappa-light-chain-enhancer of activated B cells</td>
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<tr>
<td>NOX</td>
<td>NADPH oxidase</td>
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<tr>
<td>NS1</td>
<td>Non-structural protein-1</td>
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<td>NS5B</td>
<td>RNA dependent RNA polymerase</td>
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<tr>
<td>PI3-K</td>
<td>Phosphoinositide 3-kinase</td>
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<td>Rhopty protein 18</td>
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<td>RT-PCR</td>
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<tr>
<td>RT-qPCR</td>
<td>Real time reverse transcription polymerase chain reaction</td>
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<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
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<td>siRNA</td>
<td>Small interfering RNA</td>
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<td>SKOV-3</td>
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<td>SQSTM1</td>
<td>Squestome 1</td>
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<tr>
<td>STAT</td>
<td>Signal Transducer and Activator of Transcription</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<td>TUBB3</td>
<td>β-tubulin III</td>
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<td>TUBB4</td>
<td>β-tubulin IV</td>
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<tr>
<td>TUNEL</td>
<td>Terminal deoxynucleotidyl transferase dUTP nick end labeling</td>
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<td>UTP</td>
<td>Uridine triphosphate</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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Chapter One

Introduction

1. Introduction to Guanylate-Binding Proteins

The Guanylate-Binding Proteins (GBPs) are a family of 65-67 kDa GTPases found in species, such as zebrafish, frogs, humans, mice, rats, ruminants, pigs, and chickens (1-6). GBPs are not normally expressed in resting cells but are inducible by pro-inflammatory cytokines, including IFN-α, -β, -γ, TNF-α, IL-1α and IL-β, or lipopolysaccharide (7-14). In humans there are seven hGBP paralogs (hGBP-1 to hGBP-7) and at least one pseudogene (ψ hGBP-1) located in a cluster on human chromosome 1 (15). In mice there are eleven mGBP paralogs (mGBP-1 to mGBP-11) and four pseudogenes (ψ mGBP-1 to ψ mGBP-4) and they are distributed over two chromosomes (15-17). mGBP-1, mGBP-2, mGBP-3, mGBP-5, mGBP-6, and mGBP-7, and ψ mGBP-1 are located on mouse chromosome 3. The remaining mGBP family members and three pseudogenes (ψ mGBP-2, ψ mGBP-3, and ψ mGBP-4) are located on mouse chromosome 5. Interestingly, the mGBP gene cluster on mouse chromosome 3 is syntenic to the hGBP gene cluster on human chromosome 1 (15).

A. Genomic organization of human and murine GBPs

_In silico_ analysis of the hGBP and mGBP genes revealed that they have highly similar genomic structures (15, 17). All hGBPs are encoded by genes containing eleven exons and the lengths of exons 2 to 10 are highly conserved (15). In addition, translation
of all hGBPs, except hGBP-4, begins in exon 2. The lengths of exon 1 in all hGBPs vary between 188 bp and 296 bp, except for hGBP-5. hGBP-5 has two additional exons 5’ of exon 2 which are referred to as exons 1a and 1b. In addition hGBP genes differ in the lengths of their exons 11. The genomic organization of hGBP-7 is not yet completed and therefore its exon lengths are not known (15).

Similar to the hGBP genes, all members of the mGBP family are encoded by genes containing eleven exons, except mGBP-8 which lacks exon 6 (17). Translation of all mGBPs starts within exon 2 (15, 17). All eleven members of the mGBP family have highly conserved lengths of exons 3, 4, 7, 9, and 10. The length of exon 5 is highly conserved for all mGBPs except mGBP-5. Similarly, the length of exon 8 is highly conserved in mGBPs except mGBP-4. The length of exon 6 is only conserved for mGBP-1, mGBP-2, and mGBP-5. However the lengths of exons 1 and 11 are dissimilar for all mGBPs.

(i) Promoter analysis of GBP genes

The genomic sequences from 3 kb upstream to the 5’-end of exon 1 of all human and murine GBPs were examined for potential transcription factor (TF) binding sites (7-12, 15). ISRE (Interferon-stimulated response element) and GAS (Gamma activated sequence) sequences are often found in the promoters of GBPs. Typically, an ISRE site is the binding site for the heterotrimeric protein complex containing STAT1, STAT2, and IRF-9 (IRF) (18). This heterotrimeric complex, called ISGF3, is formed in response to type I IFNs (IFNα/β). The GAS site can be bound by homodimers of STAT1 or other STATs or by IRF-1 in response to type II IFN (IFN-γ) and type III IFNs (IL-29, IL-28A
and IL-28B) (19, 20). The finding that hGBP-1, hGBP-2, and hGBP-3 contain overlapping putative ISRE and GAS sites in their promoters suggests that they could be induced by type I, type II and type III IFNs (15). The promoters of hGBP-4 and hGBP-5 each have a putative GAS site but no ISRE site, suggesting that they may be induced by IFN-γ but not by IFN-α/β. Because hGBP-1 through hGBP-5 are induced by IFN-γ in endothelial cells, this suggests that the GAS sites in hGBPs are functional (1, 16). In contrast, the promoter of hGBP-6 has an ISRE site but no GAS site, suggesting that it may be induced by IFNα/β but not by IFN-γ (15). IRF-2 sites were also identified in the promoters of hGBP-1, hGBP-2, hGBP-3 and hGBP-6 (15). Typically, IFN-induced IRF-2 competes with either IFN-γ-induced IRF-1 or IFNα/β-induced ISGF3 for binding to ISRE sites (20-23). However, co-operative activation of IRF-1 and IRF-2 increases the induction of hGBP-1 more than that induced by IRF-1 alone (24). This suggests that competition for binding of IRF-2 at ISRE sites may be inhibited by the presence of an IRF2 site in the promoters of hGBPs. A cRel/NF-κB binding site is found in hGBP-1, which co-operates with the ISRE site to induced hGBP-1 in response to IL-1β and TNF-α (1, 10, 15). An NF-κB site is also identified in the promoters of hGBP-2 and hGBP-3, suggesting that the NF-κB site may also co-operate with the ISRE site for their induction in response to IL-1β and TNF-α.

Putative overlapping ISRE and GAS elements were also identified in murine GBP members (mGBP-1 through mGBP-6) (15). Because mGBP-1 through mGBP-6 are induced by IFN-γ in endothelial cells suggests that the GAS element is functional in mGBPs (16). Similar to the hGBPs, all mGBPs contain an IRF-2 binding site. This suggests that co-operative activation of IRF-1 and IRF-2 may increase mGBP induction.
more than that induced by IRF-1 alone (24). Also, all mGBPs contain an cRel/NF-κB binding site, suggesting that the NF-κB site may also co-operate with the ISRE site for their induction in response to IL-1β and TNF-α (1, 10, 15).

(ii) *Putative orthology between GBPs*

The arrangement of GBP genes in the clusters on human chromosome 1 and murine chromosome 3 revealed that hGBP-1 and mGBP-2 have a syntenic relationship (15). Interestingly, hGBP-1 and mGBP-2 share putative ISRE, GAS, NF-κB, IRF-2, p53, and IRS sites. The arrangement of hGBP-1 and mGBP-2 in their gene cluster and their genomic organization suggests that mGBP-2 is the putative ortholog of hGBP-1.

**B. Structural organization of human and murine GBPs**

(i) *Structure of hGBP-1 protein*

The crystal structure of full length hGBP-1 in the nucleotide-free state shows that the amino-terminus has a large globular domain (LG domain) (indicated in purple in Figure 1) and the carboxy-terminus contains a series of extended α-helices (indicated in yellow and cyan in Figure 1) (25-27). These two regions are connected by a short-intermediate region (indicated in green in Figure 1). Full length hGBP-1 is 592 amino-acids long. Amino acids 6 to 278 are in the LG domain, amino acids 279 to 311 are in the short-intermediate region, and amino acids 312 to 592 are in the C-terminal region. The carboxy-terminus is composed of two bundles of 3 α-helices each, followed by two α-helices. The first helical bundle of the C-terminus contains α7, α8, and α9 helices (amino acids 311 to 403) and the second helical bundle contains α9, α10, and α11 (amino
Figure 1. Crystal structure of hGBP-1. A ribbon model of the tertiary structure of hGBP-1 is presented. The LG domain in purple color is followed by the connecting region in green color and then followed by the C-terminus containing α7 through α11 helices in yellow color and α12 and α13 helices in cyan color. Dashed lines indicate disordered regions in the molecule, which are followed by the CaaX sequence. A Ras-GBP overlay is indicated by spheres and triangle on the left side of hGBP-1. The stereotype GTPase, Ras also possesses an LG domain indicated with purple color, a connecting region indicated with green color, a C-terminus indicated with yellow and cyan colors and a CaaX sequence at the extreme C-terminus region. “Adapted by permission from Macmillan Publishers Ltd: [Nature] (B. Prakash, G. J. K. Praefcke, L. Renault, A. Wittinghofer, C. Hermann, 2000, Structure of human Guanylate-binding protein 1 representing unique class of GTP-binding proteins, Nature 403, 567-571). Copyright (2000).” Permission via Copyright Clearance Center 2013.
acids 404 to 482). The second bundle is followed by the long α12 helix (amino acids 483 to 561) and then by a short α13 helix (amino acids 562 to 588) (25). The extreme carboxy-terminus of hGBP-1 contains a CaaX motif (amino acids 589 to 592) (indicated with a blue dotted line in Figure 1).

(ii) *N-terminal GTP-binding motifs of hGBP-1*

hGBP-1 was first identified by virtue of its ability to bind to GTP-, GDP- and GMP-agarose (28, 29). hGBP-1 hydrolyzes GTP to both GDP and GMP by two consecutive cleavage reactions but it does not bind ATP, UTP, or CTP (26, 30). Together these data indicate that hGBP-1 is a GTPase. The GTPase activity of hGBP-1 increases by at least 20-fold with increasing concentration of hGBP-1, suggesting that hGBP-1 hydrolyzes GTP in a concentration-dependent manner (25, 27).

Ras, a stereotype GTPase containing four conserved amino acid sequences in its GTP-binding domain which are referred to as the G1, G2, G3, and G4 motifs were compared with GTP-binding domain of hGBP-1 (31). In Ras, the G1-motif/P-loop contains a GXXXXGK(S/T) sequence that binds the β-phosphate of GTP or GDP (25, 27, 31). In hGBP-1 this consensus GXXXXGKS sequence is located at amino acid position 45 to 52 (32). Lysine 51 of hGBP-1 interacts with the phosphate-oxygen of the non-hydrolyzable GTP analog (GppNHp) (27). In Ras, the G2switch I and G3switch II motifs are crucial for maintaining the protein either in the active state or the inactive state (33, 34). When Ras is active, GTP binds to the invariant threonine 35 within G2-motif and the glycine 60 in the consensus DXXG motif within the G3-motif. Subsequent GTP hydrolysis changes the conformation of the switch motifs so that GDP makes no contact
with the G2 motif but makes contact with the G3 motif. GDP-bound Ras is inactive as it is unable to interact with downstream targets. Similarly, the G2/s-switch I motif of hGBP-1 has a canonical threonine at position 75 that binds the γ-phosphate of GTP but makes no contact with GDP (32). hGBP-1 also has a G3/s-switch II motif containing a canonical DXXG sequence at position 97-100 which undergoes transitions between GTP- and GDP-bound states. The G4 motif of Ras contains a consensus NKXD sequence in which aspartate makes contact with the guanine base of GTP. hGBP-1 also has the canonical aspartate but it is found in the unique TLRD (tyrosine, leucine, arginine and aspartate) sequence at position 181 to 184 (35, 36). Aspartate 184 of hGBP-1 is also required for making contact with the guanine base of GTP.

(iii) Amino-acid analysis for relatedness of the human and the murine GBPs

The G1-motif is conserved in human and murine GBPs, except for mGBP-1, mGBP-4, and mGBP-10 (15, 17). However, the lysine at position 51 in the G1-motif, which is critical for binding GTP, is conserved in all GBPs. The threonine in the G2/s-switch I and the DXXG sequence in the G3/s-switch II motifs are conserved in all members of GBPs. The G4-motif containing the TLRD sequence is present in hGBP-1, hGBP-2, hGBP-3, hGBP-5, mGBP-1, mGBP-2, and mGBP-5 (15, 17). However the G4-motif of hGBP-6, hGBP-7, mGBP-4, mGBP-6 through mGBP-9, and mGBP-11 contains a TVRD (tyrosine, valine, arginine and aspartate) sequence. The G4-motif of mGBP-3 has an AVRD sequence and mGBP-10 has an IVRD sequence. Thus the aspartate residue within the G4-motif, which interacts with the guanine base is conserved for all GBPs.
C. Nucleotide-dependent oligomerization of hGBP-1

Biochemical and structural analyses of full-length hGBP-1 (hGBP-1FL) in solution indicate that when hGBP-1 is nucleotide-free it is a monomer but it can form dimers in the presence of the non-hydrolysable GTP-analog, GppNHp (25, 37). In the presence of GDP, hGBP-1FL remains in monomer. However, in the presence of GDP and aluminum fluoride, which mimics the GTP-transition state, hGBP-1 forms tetramers. Thus oligomerization of hGBP-1 is nucleotide-dependent (25, 27). Formaldehyde cross-linking of hGBP-1 in IFN-γ-treated HUVECs demonstrated the presence of dimers and tetramers in vivo (27, 37).

When GppNHp binds to hGBP-1FL, the protein dimerizes and the buried hydrophobic residues of the α6-helix of the short intermediate region are exposed. This α6-helix can then interact with the α2'-helix of the LG domain (26, 38). This suggests that the LG domain and short intermediate region play a role in dimerization. hGBP-1LG, which is a truncated version of hGBP-1 containing only LG domain and the short intermediate region (amino acid 1 to 317), also forms dimers in the presence of GppNHp (39). Structural analysis of GppNHp-bound-hGBP-1LG dimers (Figure 2a) shows that the P-loop (yellow), switch I (green), switch II (red), and aspartate 184 (cyan) remain buried within the protein (39). In this conformation, the switch I and II motifs of hGBP-1 are not accessible to possible downstream effectors. When two nucleotide-free monomeric hGBP-1FL are superimposed on dimeric hGBP-1LG, it shows that LG domain of dimeric hGBP-1LG have almost similar and compatible structure as that of LG domain of monomer hGBP-1FL (Figure 2b) (39). The only change in the LG domain of dimeric hGBP-1LG is the shifting of the backbone of α4'-helix towards the α12/13-helices at the
**Figure 2. View of the hGBP-1 dimer and its interface.** A) Figure represents the two-fold symmetry of dimeric hGBP-1\(^{LG}\) in GppNHp bound state. The cofactor Mg\(^+\) ion bound to hGBP-1\(^{LG}\) is indicated with the blue sphere. The P-loop in yellow, switch I in green, and switch II in red are buried within hGBP-1\(^{LG}\). B) Two monomers of hGBP-1\(^{FL}\) are superimposed on GppNHp bound hGBP-1\(^{LG}\) dimers. Positions of \(\alpha_{12}\) and \(\alpha_{13}\) helices of hGBP-1\(^{FL}\) are modelled with dimeric hGBP-1\(^{LG}\). C) Dimeric hGBP-1\(^{LG}\) in GppNHp bound state (represented in blue) is superimposed on dimeric hGBP-1\(^{FL}\) in GppNHp bound state (represented in yellow). The backbone of \(\alpha_{4}'\) (red arrow) shifts towards C-terminal helices \(\alpha_{12}\) and \(\alpha_{13}\) (yellow) of hGBP-1\(^{FL}\) and thus displaces \(\alpha_{12}\) and \(\alpha_{13}\) helices from the previous location. This conformational change is require to avoid steric clashes with the switch II regions of the hGBP-1 dimer. “*Adapted by permission from* Nature Publishers Ltd: [Nature] (A. Ghosh, G. J. K. Praefcke, L. Renault, A. Wittinghofer, C. Hermann, 2006 How Guanylate-binding proteins achieve assembly stimulated processive cleavage of GTP to GMP. *Nature* 440, 101-104). Copyright (2006).” Permission via Copyright Clearance Center 2013.
C-terminus of hGBP-1\(^{\text{FL}}\) (Figure 2c). This shifting of \(\alpha 4'-\)helix (red arrow) from its previous position is mandatory in dimers to avoid steric clash (red star) with the switch II motif of the dimer counterpart (red ribbon), suggesting that the shifting of \(\alpha 4'\)-helix may induce a change in the location of the C-terminal \(\alpha 12\) and \(\alpha 13\) helices of hGBP-1\(^{\text{FL}}\) dimers (37, 39).

When GDP and aluminum fluoride binds to hGBP-1\(^{\text{FL}}\), which mimics GTP-transition state, the protein forms tetramers (37). This transition from monomeric hGBP-1\(^{\text{FL}}\) to tetrameric hGBP-1\(^{\text{FL}}\) causes a change in the structure of the LG domain. Superimposition of the tetrameric hGBP-1\(^{\text{FL}}\) on the monomeric hGBP-1\(^{\text{FL}}\) (Figure 3), shows that \(\alpha 4'\)-helix of LG domain (red) of tetrameric hGBP-1\(^{\text{FL}}\) shifts away from the LG domain of monomeric hGBP-1\(^{\text{FL}}\) and interacts with the C-terminal \(\alpha 12\) and \(\alpha 13\) helices of tetrameric hGBP-1\(^{\text{FL}}\) (40). An hGBP-1 mutant which lacks both \(\alpha 12\) and \(\alpha 13\)-helices does not tetramerize in the presence of GDP and AlF\(_x\) and remains dimers (37). This suggests that the LG domain-induced structural change induced by GDP and AlF\(_x\) necessarily requires the C-terminal \(\alpha\)-helical region to regulate tetramer formation of hGBP-1.

Analyses of the biochemical properties of mutants of hGBP-1 have provided useful information about the functional role of individual residues. Serine 52 in the P-loop/G1-motif of hGBP-1 binds the cofactor Mg\(^{+}\) ion required to co-ordinate with GTP that binds to lysine at position 51 (25). Replacement of serine 52 with asparagine (S52N) impairs the binding of GppNHp to hGBP-1 (32). This suggests that S52N hGBP-1 will lack GTPase activity and it may remain as a nucleotide-free monomer in living cells. The
Figure 3. Crystal structure of hGBP-1 bound to GDP and AlF₃. Figure presents a ribbon model of the tetrameric hGBP-1FL superimposed on monomeric hGBP-1FL. The LG domain in nucleotide-free state is represented in blue color and the C-terminus containing α7 through α11 is represented in green, and α12 and α13 helices is represented in orange. The structure of hGBP-1FL in the nucleotide-free state shows no interaction of helix α4’ of LG domain (indicated in blue) with α12 and 13 helices of C-terminus. When hGBP-1FL is bound to GDP and aluminum fluoride the α4’ helix of the LG domain (indicated in red) shows a potential clash with α12 and α13 helices. “Reprinted from Science Direct, 400, Vopel, A. Syguda, N. Britzen-Laurent, S. Kunzelmann, M. B. Ludemann, C. Dovengerds, M. Sturzl, C. Hermann, Mechanism of GTPase-activity-induced self-assembly of human Guanylate-binding protein 1, 63-70. Copyright (2010) with permission from Elsevier via Copyright Clearance Center 2013.
aspartate residue at position 184 in the G4-motif of hGBP-1 is involved in the binding of the guanine base. Replacement of this aspartate 184 with asparagine (D184N) results in a 25-50 fold decrease binding of GppNHp, GDP, and GMP to hGBP-1 (32). This suggests that D184N hGBP-1 will hydrolyze GTP under physiological conditions. However, D184N hGBP-1 hydrolysis is much slower than wild-type hGBP-1 and produces less GMP (32). Additionally, D184N hGBP-1 dimerizes when bound to GTP (32).

D. Guanylate-Binding Proteins belong to dynamin superfamily

Based on the structural and biochemical properties of hGBP-1, the GBP family has been placed in the dynamin superfamily of large GTPases (41). The dynamin superfamily also includes the family of classical dynamins, the family of dynamin-like proteins, the VSP1 family, the Mx family, the ARC-5 family, the OPA family, and the Mitofusin family (42-53). Dynamin-1, a member of classical dynamin family, has an N-terminal GTP-binding domain, a middle domain, a pleckstrin homology (PH) domain, a GTPase effector domain (GED) that functions as an oligomerization domain, and a C-terminal proline rich domain (PRD) that is recognized by SH3 domain-containing proteins (41). MxA, a member of the Mx family, also contains a GTP-binding domain, a middle domain, and a GED domain but lacks PH and PRD domains (41). The structural features of hGBP-1 that are common to those of dynamin-1 and MxA include an N-terminal GTP-binding domain, a middle domain, and a C-terminal GED-like domain that is involved in oligomerization (25, 41, 54). Biochemically, the members of the dynamin superfamily of large GTPases, such as dynamin-1 and MxA, differ from members of Ras
superfamily of the small GTPases by having a lower affinity for guanine nucleotides and a high basal level of GTP hydrolysis (41, 54). Moreover, both dynamin-1 and MxA nucleotides form homo-oligomers in the absence of guanine while Ras is a monomer in the nucleotide-free state (55). Dynamin-1 and MxA assemble into rings or helices in the presence of GTP (55). This enhances GTP hydrolysis (56). hGBP-1 is a monomer when it is nucleotide free (25). It has the unique ability to hydrolyze GTP to both GDP and GMP with nearly the same affinities for GTP, GDP, and GMP (41). In contrast, dynamin-1 hydrolyzes GTP to GDP only, with lower affinity for GDP than GTP (41). Like dynamin-1 and MxA, hGBP-1 has low affinities for guanine nucleotides and a high rate of intrinsic GTPase activity, which is further stimulated by the formation of hGBP-1 dimers or tetramers (25, 30, 41, 57).

GEFs (Guanine nucleotide exchange factors) activate GTPases by stimulating the release of GDP and allowing binding of GTP (58). The GEFs for dynamin-1 are nucleotide diphosphate kinase (NDK) and Phospholipase C-γ1 (PLC-γ1) that activate dynamin-1 to mediate synaptic vesicle recycling and EGF receptor endocytosis (59, 60). However, GBPs may not require GEFs because they are induced in an already activated state by pro-inflammatory cytokines (6-14, 61-65).

GAPs (GTPase activating protein) terminate many activities of GTPases by inducing GTP-hydrolysis (58). Dynamin-1 is a mechanochemical enzyme which uses the energy from GTP hydrolysis to generate the force required for vesiculation of liposomes during endocytosis (66). It does not have an external GAP, but the interaction of the GED domain with the switch 2 region of the GTP-binding domain mediates both basal and self-assembly stimulated GTP hydrolysis (67, 68). Lysine at position 694 in the
GED domain of dynamin-1 is required for intermolecular GED–GED interactions and arginine at position 725 in the GED domain participates in GTP hydrolysis (68). Substitution of either of these residues with alanine specifically impairs self-assembly stimulated GTP hydrolysis without affecting dynamin's basal rate of GTP hydrolysis (67, 68). These data suggest that the GED domain of dynamin-1 can act as an internal GAP upon self-assembly (54, 68). This mechanism of GED-stimulated GTPase activity has also been observed for MxA, suggesting that the GED of MxA also acts as an internal GAP (69). Like dynamin-1 and MxA, hGBP-1 does not require external GAPs for GTP-hydrolysis. In hGBP-1, the α6 helix of the short intermediate region plays a critical role in GDP and GMP formation via stimulation of GTP hydrolysis. This suggests that the α6 helix can act as an internal GAP for hGBP-1 (26).

E. Isoprenylation and localization of GBPs

A CaaX motif is a consensus sequence for the addition of isoprenoids to proteins. It is composed of four amino acids that are located at the extreme carboxy-termini of proteins. It directs the post-translational modifications such as isoprenylation/lipidation, carboxy methylation and endo-proteolysis (70-72). The C in the CaaX motif stands for cysteine, aa for two aliphatic amino acids, and the X for a hydrophobic amino acid (70, 73). This hydrophobic amino acid determines the type of lipid that will added to the protein. Prenylation involves the covalent addition of either a farnesyl (15-carbon) or a geranylgeranyl (20-carbon) isoprenoid onto the cysteine of the CaaX motif via thioether linkages (74). The terminal amino acid of the CaaX for hGBP-1 and mGBP-5 is serine, which directs addition of C_{15} farnesyl (14, 15, 75). For hGBP-2, hGBP-5, mGBP-1, and
mGBP-2, the terminal amino acid is leucine, which directs addition of C_{20} geranylgeranyl lipid (15, 75-77). The remaining GBPs (hGBP-3, hGBP-4, hGBP-6 and hGBP-7, mGBP-3, mGBP-4, and mGBP-6 through mGBP-11) do not have a CaaX-motif and thus they are not prenylated.

The prenyl lipid groups of proteins can mediate their membrane association and/or their interactions with the other proteins (78, 79). For example Ras requires farnesylation to target to the plasma membrane (80, 81). hGBP-1 is found throughout the cytoplasm in a punctuate pattern and is also localized to vesicle-like structures (1). Similarly mGBP-2 is distributed in the cytoplasm and localized to vesicles (82). The identity of these vesicles-like structures is still unknown. Subcellular fractionation studies confirm that farnesylated hGBP-1, induced by IFN-γ in COS cells, is predominantly found in the cytosol with a relatively small proportion in the membrane fraction (14). However, a single point mutation in the CaaX motif of mGBP-2 that replaces cysteine with serine inhibits prenylation and prevent its localization to discrete vesicle-like structures (82). Interestingly, similar mutation of the CaaX motif of hGBP-1 also prevents its localization to Golgi compartments induced by GDP and AlF₃, suggesting that hGBP-1 requires lipid addition for its targeting to vesicles or organelles (83).
F. Functions of GBPs

(i) Antiviral activities

GBPs exhibit antiviral activity against Vesicular stomatitis virus (VSV), Encephalomyocarditis virus (ECMV), Hepatitis C virus (HCV), and Influenza A virus (IAV) (84-88). hGBP-1 and mGBP-2 inhibit VSV and ECMV replication (84, 85). Surprisingly, S52N mGBP-2 expression in NIH 3T3 cells inhibits VSV replication as efficiently as wild-type mGBP-2 (85). However, S52N mGBP-2 does not reduce ECMV replication. Together these data suggest that GTPase activity of mGBP-2 is required for the anti-viral activity against ECMV but not for the inhibition of VSV.

The expression of hGBP-1 and a splice variant of hGBP-3 (hGBP-3<sup>SV</sup>) are up-regulated in A549 lung epithelial cells infected with the influenza A virus (IAV) (88, 89). Forced expression of hGBP-1 in A549 lung epithelial cells inhibits the replication of IAV in a dose dependent manner (88). hGBP-1 is predominantly found in the cytoplasm of IAV infected cells where it influences the translocation of the non-structural NS1 protein of influenza A virus from the nucleus to the cytoplasm (88). hGBP-1 then interacts with NS1 in the cytoplasm, suggesting that binding of hGBP-1 with NS1 is required for inhibition of IAV replication. Interestingly, the residues of hGBP-1 required for binding to NS1 lie at positions 123 through 144. The expression of a mutant version of hGBP-1 (K51A), in which lysine at position 51 is replaced with alanine and is expected to lack GTPase activity, abrogates the interaction of hGBP-1 with NS1 in the cytoplasm of IAV infected cells (32, 88). This suggests that GTPase activity of hGBP-1 may be required for the inhibition of IAV. Forced expression of hGBP-1 also inhibits Hepatitis C virus (HCV) replication in Huh7 human hemochromatotic cells (87). hGBP-1 interacts
with the viral protein NS5B (RNA dependent RNA polymerase) of HCV in HEK-293 human embryonic kidney cells, suggesting that hGBP-1 may interfere with the translation of viral proteins (87). However this interaction between hGBP-1 and NS5B was absent in Huh7 human hemochromatotic cells (87). Therefore how hGBP-1 inhibits HCV replication in Huh7 cells is still unknown.

(ii) Antimicrobial activities

Some harmful bacteria and parasites live and multiply within membrane-enclosed compartments in their host cells to limit their killing by host defense (90-92). GBPs target these membrane-enclosed compartments in host cells to inhibit the growth of Chlamydia trachomatis, Salmonella enterica serovars, Toxoplasma gondii, Listeria monocytogenes, and Mycobacterium bovis BCG (93-102).

a) mGBPs inhibit Toxoplasma gondii replication

Toxoplasma gondii is an obligate parasitic protozoan which reproduces sexually in cats and asexually in humans and other warm-blooded animals, causing the disease toxoplasmosis (103). During infection in humans, T. gondii forms a unique membrane enclosed structure in the cytoplasm of host cells referred to as a parasitophorous vacuole (PV). All ten members of the murine GBPs (mGBP-1 through mGBP-10) localize around PVs within 2 hours of Toxoplasma infection in IFN-γ-treated macrophages and fibroblasts (16). Because IFN-γ actively participates in the disruption of PVs, this suggests that accumulation of GBPs around PVs may play a role in the host cellular defense. Deletion of the entire GBP cluster on chromosome 3 (mGBP-1, mGBP-2, mGBP-3, mGBP-5, mGBP-6, and mGBP-7) abolishes IFN-γ-induced disruption of PVs
(16, 98, 104). This suggests that mGBPs localize on PVs to inhibit toxoplasma replication in host cells. The localization of mGBP-1 to these PVs of *T.gondii* requires nucleotide-binding and catalytic activity of the LG domain of mGBP-1 while the isoprenylation is not required (99).

Some strains of Toxoplasma are able to limit the disruption of PVs by GBPs in IFN-γ stimulated cells. For example, the highly lethal type I strain BK of Toxoplasma inhibits the localization of mGBP-1 to their PVs (16). In contrast, the type II strain ME49 of toxoplasma which is less lethal than type I strain BK does not prevent the localization of mGBP-1 to their PVs. Interestingly, expression of certain bacterial virulence factors, such as ROP16 and ROP18 and dense granule protein GRA15, also inhibit the localization of mGBP-1 on PVs (99). ROP18 is a kinase which phosphorylates a GTPase family protein, Irgb6, at the switch I region and prevents the localization of Irgb6 on PVs (105-108). This predicts that ROP18 play a some specific role for inactivating the localization of mGBP-1 on PVs.

*Phenotype of GBP knockout mice*

To determine the role of mGBPs against *T. gondii in vivo*, the entire mGBP gene cluster on chromosome 3 (mGBP-1, mGBP-2, mGBP-3, mGBP-5, and mGBP-7) was knocked out in C57BL/6 mice by Cre/loxP recombination (98). The mGBPchr3− knockout and wild-type C57BL/6 mice were infected with *T. gondii* intraperitoneally and their survival was examined until 20 days after infection. The percentages of surviving mGBPchr3− knockout mice were lower than the wild-type mice. Also, the numbers of parasites in the spleen and mesenteric lymph nodes were higher in mGBPchr3− mice.
This suggests that the mGBP genes on chromosome 3 are involved in protection against
*T. gondii* infection. To determine if these mGBPs are involved in interferon-mediated
protection against *T. gondii*, macrophages isolated from uninfected mGBPchr3+ mice
were treated with IFN-γ for 24 hours, followed by infecting with *T. gondii* for 1, 12, 24,
36 and 28 hours and then parasitic burdens within the cells were examined (98). The
numbers of parasites in mGBPchr3+ macrophages were higher than wild-type
macrophages at 12, 24, 36, and 48 hours post infection. In addition, at 24 hours post
infection, the PV membranes surrounding the parasites were intact in IFN-γ-treated
mGBPchr3+ macrophages but showed blebbing and vesiculation in IFN-γ-treated wild-
type macrophages. This suggests that these mGBPs mediate protection against *T. gondii
during IFN-γ exposure.

To determine whether mGBP-1 targets specific strains of *T. gondii in vivo*,
mGBP-1 knockout mice were generated and their survival were monitored after infecting
them with either the highly lethal type I strain (CG-1 strain) or moderately lethal type-II
strain of *T. gondii* (109). The numbers of surviving mGBP-1 knockout and wild-type
mice were comparable when they were infected with the highly lethal type I strain of *T. gondii*. When infected with the moderately lethal type II strain, mGBP-1 knockout mice
died earlier than wild-type mice. These data suggest that mGBP-1 provides protection
against the moderately virulent strain of *T. gondii* but does not protect against the highly
lethal type I strain of *T. gondii*. To determine the cause of death of mGBP-1 knockout
mice infected with the type II strain of *T. gondii*, sections of brains were isolated from
surviving mice and their pathologies were examined after staining with hematoxylin and
eosin (H & E) (109). The brain sections of wild-type mice infected with type II strain of
*T. gondii* showed minimal focal accumulation of lymphocytes, mild perivascular cuffing, and a single tissue cyst. In contrast, the sections of brains of mGBP-1 knockout mice showed typical symptoms of encephalitis, such as elevated focal gliosis, multifocal perivascular cuffing, moderate thickening of the meninges, and multiple tissue cysts. This suggests that mGBP-1 protects the mice from encephalitis caused by type II strain of *T. gondii*. However, the type I strain of *T. gondii* was resistant to mGBP-1-mediated protection in mice (109). Interestingly, *Toxoplasma's* rhoptry kinases such as ROP5, ROP16, and ROP18 which are secreted into host cytosol upon invasion of the parasite have shown to escape interferon-mediated responses (99, 107, 110-112). To determine whether mGBP-1-mediated protection is restricted by the rhoptries of a highly virulent strain of *T. gondii*, mGBP-1 knockout and wild-type mice were infected with the type I strain of *T. gondii* deficient for either ROP5 or ROP18 and the survival of the mice were monitored (109). Lack of ROP18 delayed the death of wild-type mice as compared to mGBP-1 knockout mice. However, when infected with ROP5 deficient parasite, deaths of mGBP-1 knockout mice were comparable to wild-type mice. These data suggest that mGBP-1 is involved in protection against ROP18 deficient parasites but not involved in protecting against ROP5 deficient parasites. To confirm that mGBP-1 provides protection against ROP18 deficient *T. gondii*, bone marrow-derived macrophages (BMDM) isolated from wild-type and mGBP-1 knockout mice were treated with IFN-γ for 24 hours and then infected with the type I strain of *T. gondii* lacking either ROP5 or ROP18 for 2 hours (109). The morphologies of PV within the macrophages at 2 hours post infection were examined. In wild-type BMDM, mGBP-1 localizes on PVs of ROP5 deficient or ROP18 deficient parasites causing vesiculation of PV membranes but it is not
recruited to PVs of wild-type or complemented parasite expressing either ROP5 or ROP18. In mGBP-1 knockout BMDMs, the PVs surrounding the ROP5-deficient type I strain were also vesiculated but the PVs surrounding the ROP18 deficient type I strain were intact. These data suggest that wild-type parasite requires ROP18 to suppress mGBP-1-mediated protection against *T. gondii*. To determine if mGBP-1-mediated protection against *Toxoplasma* is inhibited by ROP18, mGBP-1 knockout and wild-type BMDMs were activated by IFN-γ for 24 hours and then infected with type I strain of *T. gondii* deficient for either ROP5 or ROP18 for 20 hours (109). Finally, numbers of parasites within the macrophages were examined at 20 hours post infection. mGBP-1 knockout BMDMs had more ROP18 deficient parasites than wild-type BMDMs. However, the numbers of ROP5 deficient parasite was not changed in mGBP-1 knockout BMDMs as compared to wild-type BMDMs. These data indicate that mGBP-1 is involved in the clearance of ROP18 deficient *T. gondii* but does not influence the clearance of ROP5 deficient *T. gondii*, confirming that wild-type *T. gondii* requires ROP18 to inhibit mGBP-1-mediated protection against the parasite.

The life cycle of *T. gondii* is divided into acute and chronic phases (113). The acute phase of *T. gondii* infection is characterized by rapid replication of lytic tachyzoites that were released from cysts. As the infection progresses, tachyzoites differentiate into slower growing bradyzoites and these later form dormant cysts in the chronic phase of infection. To determine the role of mGBP-2 during *T. gondii* infection, mGBP-2 knockout mice were infected intraperitoneally with high doses (40 cysts) of *T. gondii* strain ME49 and the survival of the mice were monitored (114). Both wild-type and mGBP-2 knockout mice showed comparable number of deaths for the first 12 days.
Examining the *T. gondii* DNA from lungs, liver, spleen, and brain of dead mice using qPCR revealed that the parasites were in the acute phase of infection. In contrast, during chronic phase of infection, mGBP-2 knockout mice showed increased mortality compared to the wild-type mice until 45 days of infection. These data suggest that mGBP-2 provides protection against the chronic phase of *T. gondii* infection but does not protect against the acute phase of infection. Examining the parasitic burdens in the brains of mice revealed that during the acute phase of infection, the numbers of parasites in mGBP-2 knockout mice were comparable to the numbers of parasites in wild-type mice. However, during the chronic phase of infection the numbers of parasites in mGBP-2 knockout mice were higher. These data confirm that mGBP-2 protects against *T. gondii* during the chronic phase of infection but not during the acute phase of infection. To determine whether mGBP-2 confers cell autonomous protection against *T. gondii*, MEFs (murine embryonic fibroblasts) and BMDMs isolated from mGBP-2-knockout and wild-type mice were infected with *T. gondii* for 32 hours and the morphologies of PVs were examined. mGBP-2 deficient fibroblasts and macrophages showed higher number of PV rosettes than wild-type fibroblasts and macrophages, indicting an initial increase in parasitic replication in the absence of mGBP-2 and suggesting that mGBP-2 mediates protection against *T. gondii* infection.

*b) hGBP-1 and hGBP-2 regulate autophagy to eliminate Chlamydial infection*

hGBP-1 and hGBP-2 localizes on Chlamydial inclusion bodies in IFN-γ-activated and *Chlamydia trachomatis* infected HeLa and THP-1 cells (93). When expressed from plasmids, ectopic hGBP-1 and ectopic hGBP-2 also localize on Chlamydial inclusions and inhibit the replication of *Chlamydia trachomatis* (93). D184N hGBP-1 which is
expected to decrease GTP hydrolysis, is sufficient for translocation of hGBP-1 to *Chlamydial* inclusions but is unable to inhibit the replication of *Chlamydia* (93). This suggests that after localization of hGBP-1 on *Chlamydial* inclusions, it requires the LG domain to inhibit *Chlamydia* growth.

hGBP-1 and hGBP-2 facilitate fusion of Chlamydial inclusions to autolysosomes, suggesting that they may regulate autophagy in host cells for elimination of bacterial growth (94). Generally pathogens enter into host cells either through active invasion or via engulfment and formation of intracellular vacuoles called phagosomes. The phagosomes containing intracellular pathogens are sequestered into double membrane vesicles called autophagosomes. The autophagosomes are then fused with lysosomes to form autolysosomes, which contain a range of acidic hydrolases to facilitate killing of the pathogens (115). During the process of autophagy, three autophagy-related proteins (ATGs) form a conjugate called the ATG5-ATG12-ATG16 complex which then facilitates the fusion of cytosolic LC3-I protein with phosphatidyl-ethanol-amine to form LC3-II (115, 116). LC3-II is found on the autophagosomes, indicating that it is required for autophagy. Knockdown of hGBP-1 or hGBP-2 results in monomeric ATG5 and ATG12 rather than the formation of the ATG5-ATG12 complex in IFN-\(\gamma\)-treated macrophages were infected with *Chlamydia* (94). This suggests that hGBP-1 and hGBP-2 may regulate autophagosomal formation for the inhibition *Chlamydia trachomatis*. The sequestosome 1, also referred to as p62/SQSTM1, is recruited in the autophagosomes (117). SQSTM1 delivers ubiquitinated proteins into autophagosomes and these ubiquitinated proteins are then converted to proteolytic proteins for degradation of the pathogens (100-102). Typically, in later stages of autophagy the SQSTM1 is itself
degraded (118, 119). Interestingly, SQSTM1 accumulates when *Chlamydia* infects the macrophages but IFN-γ-treatment reduces the level of SQSTM1 in those *Chlamydia* infected macrophages (94). Knockdown of hGBP-1 or hGBP-2 abolishes the reduction of SQSTM1 in IFN-γ-treated macrophages. Together these data suggest that hGBP-1 and hGBP-2 by a IFN-γ-induced autophagic pathway regulates the killing of *Chlamydia trachomatis*.

c) *mGBPs assemble autophagic and oxidative complexes to eliminate Listeria monocytogenes and Mycobacterium bovis*

*Listeria monocytogenes* (*Lm*) and *Mycobacterium bovis* (*Mb*) are intracellular bacteria that cause virulent and lethal diseases (120, 121). The expression of mGBP-1 through mGBP-10 are up-regulated in the spleens and livers of *Lm* infected C57BL/6 mice, predicting a possible role for these mGBPs in defense against *Lm* (16). IFN-γ-treatment facilitates the lysosomal degradation of *Lm*- and *Mb*-containing vacuoles (121-123). All ten members of the mGBPs induced by IFN-γ are assembled on *Lm*- and *Mb*-containing vacuoles in RAW264.7 murine macrophages (100). siRNA silencing of mGBP-1, mGBP-6, mGBP-7, and mGBP-10 abolishes IFN-γ-mediated killing of *Lm* or *Mb* (100). S52N mGBP-1 reduces the killing of *Listeria* in infected cells, suggesting that the catalytic activity of mGBP-1 is required for the inhibition of *Listeria* (100).

mGBP-7 co-localizes with ATG4 at the site where LC3-II is present in *Mb*-infected macrophages (100). Typically, the ATG4 protein cleaves precursor LC3 protein at its C-terminus to generate LC3-I which is then lipidated to form LC3-II (116, 124). The LC3-II and ATG4 protein complex are then recruited to autophagosomal membranes
Knockdown of mGBP-7 abrogates the co-localization of ATG4 with LC3-II on Mb-containing vacuoles in IFN-γ-treated RAW264.7 cells (100). Together these data suggest that mGBP-7 may facilitate autophagy for killing of Mb. mGBP-1, which is also found on Mb-containing vacuoles, binds p62/SQSTM1 in Mb-infected cells (100). This suggests that mGBP-1 may also facilitate autophagy to kill Mb. Knockdown of mGBP-1 abolishes IFN-γ-mediated reduction of SQSTM1 in Mb infected macrophages (100). This suggests that mGBP-1 regulates IFN-γ-mediated autophagic pathway for the elimination of Mycobacterium infection.

Formation of cytotoxic gas is an important cell-autonomous defense against pathogens. The generation of superoxide by the family of NADPH oxidases (NOX1 through NOX5) causes microbicidal activity in cells (125). NADPH oxidase is composed of 5 subunits. One subunit is the catalytic NOX2/gp91Phox subunit, another is the membrane-associated, regulatory p22phox subunit, and the remaining three are cytosolic regulatory subunits, called p40Phox, p47Phox, and p67Phox (125). IFN-γ induced mGBP-7 plays a role in the recruitment of NADPH oxidase to Lm- or Mb-containing vacuoles, indicating that mGBP-7 may be involved in regulating the oxidative killing of pathogens (100). Typically, the p22Phox subunit of NADPH oxidase forms a complex with gp91Phox, called flavocytochrome b558 while phosphorylated p47Phox forms a complex with p67Phox, and p40Phox (123, 125, 126). Flavocytochrome b558 when assembled with the p47Phox-p67Phox-p40Phox complex is recruited to pathogenic vacuoles. mGBP-7 acts as a bridging protein between p67Phox and gp91Phox of NADPH oxidase subunits for the assembly of its subunits to Lm- or Mb-containing vacuoles (100). Knockdown of mGBP-7 in macrophages inhibits the IFN-γ-induced $O_2^-$.
production and diminishes the IFN-γ-mediated targeting of p67Phox to Lm- or Mb-containing vacuoles. Knockdown of other mGBPs, such as mGBP-1 and mGBP-10, does not abrogates INF-γ induced production of O$_2^-$ in listeria and mycobacterium infected cells (100). Together these data suggest that mGBP-7 is crucial for mediating IFN-γ-induced oxidative protection against Listeria and Mycobacteria infection.

(iii) GBPs inhibit apoptosis of intestinal epithelial cells and regulate epithelial barrier

The intestines of human and murine newborns are sterile at birth but later become populated with commensals that include E. coli. In the intestinal tract of 2-week old mice, E.coli induces the expression of both IFN-α2A and mGBP-1 (127). Ex-vivo analysis of 2-week old murine intestinal epithelia reveals that staurosporine-induced apoptosis is decreased when mice were fed E. coli. This predicts that the induction of mGBP-1 by IFN-α2A may inhibit apoptosis of intestinal epithelium. Consistent with this, IFN-αA inhibits staurosporine-induced apoptosis of FHs Int 74, immature human epithelial cells, and up-regulates hGBP-1 expression (127). The IFN-αA protection against staurosporine-induced apoptosis was abolished when hGBP-1 was knocked down in IFN-αA-treated FHs Int 74 cells. This suggests hGBP-1 plays a critical role in intestinal epithelial homeostasis.

IFN-γ also induces hGBP-1 in another three human intestinal epithelial cell lines (T84, SK-CO15, and HT29). hGBP-1 co-localizes with CAR (Coxsackie and adenovirus receptor) at the tight-junctions of SK-CO15 cells (128). CAR is a type I membrane receptor which mediates cell-cell adhesion of intestinal epithelium (129, 130). Its expression reduces the permeability of ions and molecules across the epithelia.
Interestingly, IFN-γ in TNF-α-treated SK-CO15 cells prevents permeability of intestinal epithelium, which is indicated by an increase of transepithelial electrical resistance (TER) (128). TER is the method for in vitro measurement of permeability at the tight junctions of cells. Knockdown of hGBP-1 reduces the IFN-γ-mediated increase in TER in TNF-α-treated SK-CO15 cells (128). This reduced TER was increased by the addition of Z-VAD, a caspase inhibitor, in IFN-γ- and TNF-α-treated SK-CO15 cells. Together these data suggest that hGBP-1 regulates intestinal epithelial barrier. Knockdown of hGBP-1 enhances TNF-α-induced apoptosis in IFN-γ-treated SK-CO15 cells (128). This suggests that hGBP-1 may inhibit epithelial permeability by inhibiting epithelial apoptosis.

(iv) GBPs inhibit cell spreading

Forced expression of hGBP-1 in human umbilical vein endothelial cells (HUVECs) up-regulates integrin-α4 (ITGA4) and inhibits the spreading of HUVECs on fibronectin (62). This predicts that hGBP-1 may mediate the inhibition of cell spreading through up-regulation of integrin-α4 (ITGA4). TNF-α and IL-1β, which modestly induce hGBP-1 in HUVECs, also up-regulate integrin-α4 and inhibit cell spreading (62). In contrast, IFN-γ, which robustly induces hGBP-1, does not up-regulate integrin-α4 but still inhibits cell spreading of HUVECs (62). The mechanism by which IFN-γ inhibits the spreading of HUVECs is not fully understood.

IFN-γ treatment of NIH 3T3 murine fibroblast cells induces mGBP-2 and inhibits their spreading on fibronectin (131). Forced expression of mGBP-2 in NIH 3T3 cells does not induce integrin-α4 but inhibits cell spreading. mGBP-2 or IFN-γ treatment inhibit integrin-mediated activation of the small GTPase, Rac1. This reduction of active Rac may slow down the formation of lamellipodia needed for cell spreading (132, 133).
Additionally, mGBP-2 or IFN-γ treatment inhibits PDGF-induced spreading of NIH 3T3 cells and attenuates Rac activation. Together these data suggest that IFN-induced mGBP-2 inhibits Rac activation by both integrin-engagement and growth factor treatment. However, mGBP-2 does not interact with Rac itself, suggesting that mGBP-2 inhibits a pathway to Rac activation. PI3-K, an important component of a signaling cascade that can lead to Rac activation upon integrin-engagement or PDGF treatment, is inactivated by mGBP-2 during cell spreading (131, 132, 134). mGBP-2 is found within the protein complex containing p110, the catalytic subunit of PI3-K. This suggests that the interaction of mGBP-2 with p110 causes the inactivation of PI3-K during spreading. Together the data indicate that the inhibition of Rac activation by mGBP-2 is accompanied by inactivation of PI3-K. The mechanism of how mGBP-2 inhibits Rac activation during cell spreading is not clear, but possibly the interaction of mGBP-2 with PI3-K may inhibit the binding of Rac to PI3-K.

\(v\) \textit{The expressions of GBPs and matrix metalloproteinases are correlated}

To date more than 20 metalloproteinases (MMPs) have been identified in humans (135). MMPs belong to a family of calcium-dependent, zinc-containing endopeptidases. They are capable of tissue remodeling and degrading extracellular matrix that includes collagen, gelatin, matrix glycoprotein, proteoglycans, and stromelysin (135). Depending on their substrate, MMPs are categorized as collagenases, gelatinases, stromelysins, matrilysins, metalloelastases, and membrane-type matrix metalloproteinases (136). MMP-1 belongs to the collagenases because it is able to cleave collagen type I, II, and III. MMP-9 is a gelatinase because it can degrade gelatin (137, 138). Both MMP-1 and MMP-9 play pivotal roles in cell migration, invasion, and angiogenesis (139).
a) *hGBP-1 suppresses angiogenesis and MMP-1 expression in endothelial cells*

Ectopic expression of hGBP-1 or treatment with IL-1β, TNF-α, or IFN-γ inhibits bFGF- and VEGF-induced MMP-1 expression in HUVECs (140). This reduction of MMP-1 by hGBP-1 correlates with reduced cell invasion and migration through collagen, suggesting that hGBP-1 inhibits transmigration of bFGF- and VEGF-treated endothelial cells via the inhibition of MMP-1 expression (140). The expression of hGBP-1 in HUVECs also reduces capillary formation in matrigel, suggesting that hGBP-1 inhibits MMP-1-induced angiogenesis of endothelial cells (140). Expression of D184N hGBP-1 in HUVECs does not inhibit bFGF- and VEGF-induced MMP-1 expression or decrease tube formation in matrigel. Additionally, the truncated version of hGBP-1, containing only C-terminus does not inhibit bFGF- and VEGF-induced MMP-1 expression.

b) *EGF-induced hGBP-1 expression up-regulates MMP-1 and increases cell invasion of glioblastoma*

The expression of hGBP-1 and MMP-1 are induced by epidermal growth factor (EGF) in EGFR-expressing glioblastomas (64). Knockdown of hGBP-1 in glioma cells inhibits EGF-induced MMP-1 expression and cell invasion. The invasive behavior is also observed *in vivo* when hGBP-1-expressing SNB19 human glioma cells are implanted into mice brains. This hGBP-1-expressing SNB19 cells increases diffuse tumor mass formation with invasive lesions at periventricular region of the mice brains as compared to SNB19 human glioma cells in which hGBP-1 is knocked-down. Together these data suggest that hGBP-1 through MMP-1 enhances glioma cell invasion. Expression of hGBP-1 containing only LG domain in glioma cells is unable to induce MMP-1.
However, expression of hGBP-1 containing only C-terminal helical domain in glioma cells is able to induce MMP-1 expression and also induces cell invasion. Together these data suggest that GTPase activity of hGBP-1 may require for MMP-1-induced glioma cell invasion.

c) mGBP-2 suppresses TNF-α induced MMP-9 expression in fibroblasts

Ectopic expression of mGBP-2 or IFN-γ treatment suppresses TNF-α-induced MMP-9 expression in NIH 3T3 murine fibroblasts (141). The inhibition of TNF-α-induced MMP-9 is restored by the knockdown of mGBP-2 in IFN-γ-treated fibroblasts. This shows that mGBP-2 is involved in the IFN-γ-mediated inhibition of TNF-α-induced MMP-9 expression. Induction of MMP-9 by TNF-α in a human cardiac myofibroblast cell line correlates with enhancement of cell invasion without influencing their migration (142). Together these data suggest that mGBP-2 may inhibit cell invasion of murine fibroblasts via suppression of MMP-9 expression. This inhibition of MMP-9 induction by mGBP-2 in TNF-α-treated cells is mediated via inhibition of NF-κB binding to the MMP-9 promoter (141).

2. Introduction to the Taxane family of drugs

The taxane family of drugs includes paclitaxel, docetaxel, cabzitaxel, abraxane, tesetaxel, opaxio, endoTAG, larotaxel, genexol-PM, and taxoprexin (143, 144). Paclitaxel was the first taxane drug identified. It is extracted from the Pacific yew tree, Taxus brevifolia (145). Paclitaxel can heal both leukemias and solid tumors (146). Because Taxus brevifolia is an exhaustible source of paclitaxel, a new semisynthetic
analogue called docetaxel (taxotere) was developed. Docetaxel is synthesized from 10-deacetyl baccatin III, which is extracted from the needle of the European Yew, *Taxus Baccata*, and the Himalayan yew, *Taxus yunnanensis* (147).

Both paclitaxel (C$_{47}$H$_{51}$O$_{14}$) and docetaxel (C$_{43}$H$_{55}$NO$_{14}$) have a similar pentamethyl tricyclopentadecane skeleton, called taxane rings, but they differ in their chemical composition at two positions (Figure 4) (148-150). The carbon-10 of paclitaxel has an acetate ester group whereas docetaxel has a hydroxyl group at this position. As a consequence, docetaxel is more soluble in water than paclitaxel. The second difference between paclitaxel and docetaxel is present at the 3’-position of the lateral phenylpropionate chain. At this position, docetaxel has a tert-butyl carbamate ester and paclitaxel has a benzamide phenyl group. As a consequence, docetaxel can be easily hydroxylated resulting in greater solubility in aqueous solution.

A. **Action of taxane drugs**

(i) *Taxanes modify microtubule dynamics*

Microtubules are a component of the cytoskeleton, which is involved in the development and maintenance of cell shape, cell reproduction and division, cell signaling, and the transport of vesicles within cells (151-155). Each microtubule is a long hollow cylinder with a diameter of ~24 nm. It is composed of heterodimers of α- and β-tubulins (156). The heterodimeric subunits are arranged head to tail into protofilaments. Thirteen protofilaments are arranged laterally to generate the mature microtubule in eukaryotes. Subunits are preferentially added at one end of the microtubule referred to as
Figure 4. Structure of paclitaxel and docetaxel. Paclitaxel and docetaxel both possess taxane rings that differ in chemical composition at two positions, indicated by R and R’ in the figure. R is located at the 3’-position of the lateral phenylpropionate side chain. At this position Ph indicates a benzamide phenyl group in paclitaxel and tBuO indicates a tert-butyl carbamate ester in docetaxel. R’ is located at the C-10 position. At this position, Ac indicates an acetate ester group in paclitaxel and H represents a hydrogen in docetaxel. “Reprinted from ChemMedChem by EU ChemSoc, (Robert D. Winefield, Ruth A. Entwistle, Travis B. Folanda, Gerald H. Lushington, and Richard H. Himesa. (2008) Differences in paclitaxel and docetaxel interactions with tubulin detected by mutagenesis of yeast tubulin 3(12), 1844-1847). Copyright (2008) with permission of WILEY VCH via Copyright Clearance Center 2013.”
the plus end of microtubule while the another end of the microtubule is referred to as the minus end. In microtubules, the β subunits are exposed at the plus end and α subunits are exposed at the minus end. The addition of tubulin heterodimers to microtubules continues until the concentration of free tubulin α and β heterodimers drops below critical concentration. Below the critical concentration the tubulin subunits disassemble from the microtubules causing shrinking/shortening of microtubules. In mature microtubules, the plus end can alternate between periods of elongation by addition of tubulin heterodimers and periods of shortening by disassembly of tubulin heterodimers (157-159). This phenomenon is called dynamic instability.

Paclitaxel binds the N-terminal domain of β-tubulins both at the plus end and in the lumen of microtubules (160-162). At a stoichiometry of 1 mole of paclitaxel to per mole of assembled α and β tubulin heterodimers, paclitaxel inhibits the rate of tubulin heterodimer removal and increases the mass of the microtubules (163). In Caov-6 ovarian and A-498 kidney cancer cells, paclitaxel inhibits the shortening of microtubule length during the disassembly stage while it increases microtubule length during assembly stage of tubulins (164). Together these data suggest that paclitaxel stabilizes microtubules by inhibiting their depolymerization and stimulating their polymerization. Similar to paclitaxel, docetaxel also promotes the assembly of microtubules and inhibits their depolymerization (165-167).

(ii) Paclitaxel induces abnormal spindle formation

Microtubule dynamics play an important role in cell cycle (152-155). During interphase, the microtubules radiate from a central site located beside the nucleus called
the microtubule-organizing center (MTOC), associated with the centrosome in eukaryotes (168, 169). In the MTOC, the plus ends of microtubules point outward from centrosome to the cell periphery while the minus end of microtubules lie in or near the centrosome. During interphase, the centrosome divides into two centrosomes and they remain together at one side of the nucleus (170). Also in interphase, the cytoplasmic microtubules are organized into 3 to 5 longitudinal arrays of antiparallel overlapping microtubules called microtubule bundles (171, 172). These microtubule bundles run in cytoplasm of the cells, forming a basket around the nucleus. They are responsible for proper positioning of the nucleus. When the cells enter mitosis, the two centrosomes separate to opposite sides of the nucleus. Both centrosomes radiate microtubules towards the center of the cells forming a bipolar spindle (170, 173). Additionally, two astral microtubules point outward from the two poles of bipolar spindle (174). As mitosis proceeds, the sister chromatids are align at the equatorial/metaphase plate on bipolar spindle (175). Later these sister chromatids are separated which then moves to the opposite poles of the spindle (170, 175).

Paclitaxel treatment of the leukemic cell lines LC8A, K562, Daudi, and HL-60, increases the number of cytoplasmic microtubule bundles during interphase (176). During mitosis, paclitaxel induces the formation of more than two astral microtubules. Moreover, paclitaxel treatment of HeLa cells results in abnormal spindle morphologies, which include either localizing the sister chromatids at the poles of the bipolar spindle rather than at the metaphase plate or forming monopolar microtubules containing ball-shaped aggregates of chromosome (177). The monopolar spindle consists of a half
spindle with one astral microtubule on which the sister chromatids fail to segregate (178, 179). In Caov-3 and A-498 cells, paclitaxel also induces formation of multipolar spindles which have more than two spindle poles (164). In normal BSC1 African green monkey kidney cells, paclitaxel induces the formation of multiple asters and multipolar spindles (180). Together these data suggest that paclitaxel causes changes in the organization of microtubules.

(iii)  Paclitaxel induces mitotic arrest and apoptosis

Paclitaxel induces the death of a variety of cell types (164, 181, 182). Treatment of HeLa cells with low concentrations of paclitaxel (10 mM) for 20 hours causes mitotic arrest at the metaphase/anaphase boundary (181). Following removal of paclitaxel, the cells did not resume proliferation. Instead they abnormally exited from mitosis and entered a multinucleated interphase-like state. The cells eventually died by apoptosis. Similarly, paclitaxel treatment of Caov-3 ovarian and A-498 kidney cancer cells causes mitotic arrest and after removal of paclitaxel these cells also abnormally exit from mitosis and eventually die by apoptosis (164). In contrast, HL-60 acute myeloid leukemia cells exposed to either low concentrations (10 nM) of paclitaxel for 24 hours or high concentrations of paclitaxel (60 nM) for 1 hour failed to arrest in mitosis but still underwent apoptosis after the removal of paclitaxel (182). Together these data suggest that mitotic arrest is not a prerequisite for paclitaxel-induced apoptosis. Surprisingly, treatment of HL-60 cells with low concentrations of paclitaxel (10 nM) for 12 hours resulted in a block of one subpopulation of cells at the G2/M interface and another subpopulation at S-phase. All of the HL-60 cells eventually died by apoptosis. This
suggests that there may be more than one mechanism by which paclitaxel induces apoptosis.

(iv) Paclitaxel and Docetaxel

Based on paclitaxel structural activity relationship (SAR), the free hydroxyl group at C-13, the esters group at C-2 and oxatene moiety at C-4 and C-5 are essentially required for microtubule stabilization (Figure 4) (183). The similar chemical composition of docetaxel, suggests that paclitaxel and docetaxel may use similar mechanism to promote the assembly of microtubules and inhibit their depolymerization. However, paclitaxel reduces the number of protofilaments in a microtubule from 13 to 12 while docetaxel does not (161). Docetaxel has a higher affinity for β-tubulin than paclitaxel, which may be due to its higher solubility (161). This does not necessarily mean that docetaxel produces greater cytotoxic effects. For example, paclitaxel more potently inhibits the proliferation of HCT116 cells, while docetaxel more potently inhibits the proliferation of HeLa cells (184, 185). Docetaxel is completely lethal to HeLa cells at interphase and has less effect on the cells in mitosis. In contrast, paclitaxel gradually increases killing of HeLa cells as they progressed through interphase to mitosis (185). This suggests that paclitaxel and docetaxel may have different mode of action to induce cells death.

B. Resistance to taxane-induced cell death

The clinical utility of taxane drugs is limited by the development of resistance. Intrinsic and acquired drug resistance are believed to cause treatment failure in patients
with cancer (186). A better understanding of the mechanisms of resistance to paclitaxel may provide us with a method to overcome the failure of chemotherapy.

There are a number of proposed mechanisms for taxane resistance. Because taxane targets β-tubulin in microtubules, this suggests one mechanism for taxane resistance may be the inhibition of binding of taxane to β-tubulin. There are six isotypes of β-tubulins (β-I, II, III, IVa, IVb, and VI) in eukaryotic cells (187-190). A549 human lung cancer cells that acquired resistance to paclitaxel displayed elevated levels of β-1, β-II, β-III, β-IVa tubulins (191). Additionally, four ovarian tumors that were resistant to chemotherapy which included paclitaxel treatment had increases the levels of β-I, β-III, β-IVa tubulins as compared to the primary ovarian tumors which were isolated from the patient prior to treatment (191). β-III (TUBB3) or β-IV (TUBB4) tubulins also reduces paclitaxel-induced suppression of microtubule dynamics (192). Knockdown of TUBB3 in H460 human lung cancer cells abolishes paclitaxel-induced stabilization of microtubule dynamics and mitotic arrest at that G2/M interphase and finally results apoptosis of H460 cells (193). Together these data suggest that up-regulation of TUBB3 could be a possible biomarker for paclitaxel resistance.

Paclitaxel resistance may also be mediated by increased levels of membrane transporters. P-glycoprotein (MDR1) is an ATP driven membrane efflux pump which when up-regulated may maintain intracellular drug concentrations below cytotoxic levels (194, 195). The MDR1 gene is frequently up-regulated in paclitaxel-resistant cells and in tumors from those patients who received prior treatment of either docetaxel or paclitaxel (196, 197). Up-regulation of MDR1 in nine MES human sarcoma clones that acquired resistance to paclitaxel also resulted in resistance to docetaxel (196, 197). The levels of
MDR1 in these clones were directly correlated with the levels of resistance to paclitaxel and/or docetaxel. This suggests that up-regulated expressions of MDR1 may result in inhibition of the cytotoxic effects of taxanes.

hGBP-1 is another gene whose expression is up-regulated with paclitaxel-resistance in some ovarian and breast cancer cells (198, 199). In the present study, the contribution of hGBP-1 in inhibiting paclitaxel-induced apoptosis has been explored. Additionally, the tumor samples from patients who were given prior chemotherapy with paclitaxel or docetaxel are also evaluated for up-regulation of hGBP-1. In a published study, the expression of TUBB3 is found inversely correlated with the expression of hGBP-1 in ovarian carcinoma cells (200). The present study also determines whether the expressions of hGBP-1 and TUBB3 are correlated in tumor samples from patients who were given prior chemotherapy with paclitaxel or docetaxel.

3. Binding partners of GBPs

To identify proteins that may facilitate hGBP-1-mediated inhibition of cell spreading, Flag-tagged-hGBP-1 was immunoprecipitated from HeLa cells that were plated overnight and its potential binding partners were analyzed via MALDI-TOF mass spectrometry and peptide mass finger printing (201). The nine putative interacting proteins identified were actin, tubulin B-polypeptide (TUBB), heat shock protein 90-beta (HSP90B), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), lactate dehydrogenase A (LDH A), 60S ribosomal proteins L5 (RPL5), ADP-ATP-carrier protein liver isoform
T2 (SLC25A6), 14-3-3 protein epsilon (YWHAE), and peroxiredoxin 1 (PRDX1) (Table 1) (201). Only the potential interaction between actin and hGBP-1 was further explored. To determine the effect of hGBP-1 on the actin cytoskeleton, the F-actin (filamentous actin) in GFP-tagged-hGBP-1-expressing HeLa cell was stained with fluorescent-labelled phalloidin and the morphology of F-actin was examined (201). hGBP-1 co-localizes with actin both at the cell membrane and within the cytoplasm but disrupts the parallel orientation of F-actin stress fibers in the cytoplasm. Also, IFN-γ treatment disrupts the parallel orientation of cytoplasmic stress fibers in HeLa cells. Knockdown of hGBP-1 during IFN-γ treatment in HeLa cells restores the parallel orientation of F-actin stress fibers. This suggests that hGBP-1 is involved in the remodeling of the actin cytoskeleton (201). Actin can exist as either monomeric G-actin (globular actin) or as a part of linear polymers of G-actin called F-actin (filamentous actin) (202). To confirm that hGBP-1 binds to F-actin, monomeric G-actin was polymerized to F-actin in the absence and presence of purified recombinant hGBP-1 in vitro and then the resulting F-actin was sedimented by ultracentrifugation (201). The sedimented F-actin was size fractionated on SDS-PAGE and stained with coomassie-blue (201). Bands of both actin and hGBP-1 were detected on the gel, suggesting that hGBP-1 forms a complex with F-actin. To determine whether hGBP-1 binds to G-actin, G-actin was incubated with increasing concentrations of recombinant hGBP-1 in actin depolymerizing buffer for 2 hours and then soluble G-actin was separated from F-actin by centrifugation (201). G-actin in the supernatant was size fractionated on native PAGE and analyzed by western blotting. The actin antibody recognized a higher molecular weight species in the presence of hGBP-1 and the intensity of this band was greatest at the highest concentration of hGBP-1. This
TABLE 1

1. Cytoskeleton remodeling
   ▪ hGBP-1 and actin in HeLa cells, DLD-1 cells, and T-lymphocytes (201, 203).
   ▪ hGBP-1 and β-II spectrin in T-lymphocytes (203).
   ▪ mGBP-2 and PI3-K in NIH 3T3 cells (131).
   ▪ hGBP-1 and plastin-2 in T-lymphocytes (203).
   ▪ hGBP-1 and TUBB in HeLa cells and T-lymphocytes (201, 203).

2. Autophagy
   ▪ mGBP-7 and ATG4 in RAW264.7 cells infected with Listeria monocytogenes or Mycobacterium bovis (100).
   ▪ mGBP-1 and p62/SQSTM1 in RAW264.7 cells infected with Listeria monocytogenes or Mycobacterium bovis (100).

3. Anti-microbial/Anti-viral
   ▪ mGBP-7 and p67Phox subunit of NADPH in RAW264.7 cells infected with Listeria monocytogenes or Mycobacterium bovis (100).
   ▪ mGBP-7 and p91Phox subunit of NADPH in RAW264.7 cells infected with Listeria monocytogenes or Mycobacterium bovis (100).
   ▪ hGBP-1 and NS1 of Influenza A virus (88).

4. Viral protein
   ▪ hGBP-1 and NS5 of Hepatitis C virus (87).

5. Other interacting proteins
   ▪ hGBP-1 and ENO1 in T-lymphocytes (203).
   ▪ hGBP-1 and EPRS in T-lymphocytes (203).
   ▪ hGBP-1 and GAPDH in HeLa cells (201).
   ▪ hGBP-1 and HSP90A in T-lymphocytes (203).
   ▪ hGBP-1 and HSP90B in HeLa cells (201).
   ▪ hGBP-1 and IARS in Jurkat T-lymphocytes (203).
   ▪ hGBP-1 and LDHA in HeLa cells (201).
   ▪ hGBP-1 and MYLC2B in T-lymphocytes (203).
   ▪ hGBP-1 and PRDX1 in HeLa cells (201).
   ▪ hGBP-1 and RPL5 in HeLa cells (201).
   ▪ hGBP-1 and SLC25A6 in HeLa cells (201).
   ▪ hGBP-1 and STOML2 in T-lymphocytes (203).
   ▪ hGBP-1 and YBX1 in T-lymphocytes (203).
   ▪ hGBP-1 and YWHAE in HeLa cells (201).

6. Interacting protein kinases
   ▪ hGBP-1 and AXL in vitro (200).
   ▪ hGBP-1 and BTK in vitro (200).
   ▪ hGBP-1 and CAMK2D in vitro (200).
   ▪ hGBP-1 and CHEK2 in vitro (200).
   ▪ hGBP-1 and CSNK1E in vitro (200).
   ▪ hGBP-1 and CSNK1G2 in vitro (200).
   ▪ hGBP-1 and EPHA3 in vitro (200).
   ▪ hGBP-1 and FES in vitro (200).
   ▪ hGBP-1 and KDR in vitro (200).
   ▪ hGBP-1 and MAP2K2 in vitro (200).
   ▪ hGBP-1 and NEK6 in vitro (200).
   ▪ hGBP-1 and NTKR3 in vitro (200).
   ▪ hGBP-1 and PAK6 in vitro (200).
   ▪ hGBP-1 and PBK in vitro (200).
   ▪ hGBP-1 and PIM1 in vitro (200).
   ▪ hGBP-1 and PLK1 in vitro (200).
   ▪ hGBP-1 and TSKK1 in vitro (200).
   ▪ hGBP-1 and WIBG in vitro (200).
**Table 1. Putative binding partners of GBPs.** Proteins that putatively interact with GBPs are listed in this table. The potential interacting proteins are grouped by their function and within these groups the interacting proteins are alphabetized. The abbreviations for interacting partners are: PI3-K, phosphoinositides 3 kinase; TUBB, tubulin beta polypeptide. ATG4, autophagy related proteins; SQSTM1, sequestome 1; NS1, non-structural protein 1; NS5, non-structural protein 5; ENO1, alpha-enolase, EPRS, bifunctional aminoacyl-tRNA synthetase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSP90A, heat shock protein 90-alpha; HSP90B, heat shock protein 90-beta; IARS, isoleucyl-tRNA synthetase; LDHA, lactate dehydrogenase A; MYLC2B, myosin regulatory light chain; PRDX1, peroxiredoxin 1; RPL5, 60S ribosomal proteins L5; SLC25A6, ADP-ATP-carrier protein liver isoform T2; STOML2, stomatin-like protein; YBX1, nuclease-sensitive element-binding protein 1; YWHAE, 14-3-3 protein epsilon, AXL, tyrosine protein kinase receptor UFO; BTK, bruton tyrosine kinase; CAMK2D, calcium/calmodulin-dependent protein kinase type II delta chain; CHEK2, checkpoint kinase 2; CSNK1E, casein kinase 1, epsilon; CSNK1G2, casein kinase 1, gamma 2; CAMK2D, calcium/calmodulin-dependent protein kinase type II delta chain; EPHA3, ephrin type-A receptor 3; FES, Feline sarcoma oncogene; KDR, kinase insert domain receptor; MAP2K2 stands for mitogen-activated protein kinase kinase 2; NEK6, serine/threonine kinase NEK6; NTKR3, neurotrophic tyrosine kinase, receptor, type 3; PAK6, p21 protein (Cdc42/Rac)-activated kinase 6; PBK, PDZ binding kinase; PIM 1, proto-oncogene serine/threonine kinase PIM1; PLK1; polo like kinase 1, TSKK1, human spinster homologue 1 (Drosophila); WIBG, human within Bgen homolog (Drosophila).
suggests that G-actin forms a complex with hGBP-1. Binding of hGBP-1 to G-actin may lead to reduction of the G-actin pool available for actin polymerization. To examine if hGBP-1 inhibits actin polymerization, monomeric G-actin was polymerized to F-actin in the presence and absence of purified recombinant hGBP-1 \textit{in vitro} and the resulting F-actin was deposited on MgCl$_2$-pretreated mica (201). After drying the F-actin on MgCl$_2$-pretreated mica, the lengths of the actin filaments were analyzed by atomic force microscopy. The lengths of actin filaments in the presence of hGBP-1 were shorter as compared to those in the absence of hGBP-1. This suggests that hGBP-1 inhibits actin polymerization. Thus inhibition of actin polymerization by hGBP-1 may result in restricting cellular movements during spreading. Interestingly, mGBP-2 inhibits fibroblast spreading by inhibiting the activation of Rac, a master regulator of the actin cytoskeleton (Table 1) (131). Altogether the data demonstrate that the family of GBPs can regulate the cytoskeleton.

To determine whether hGBP-1 plays a role in remodeling of the T-cell cytoskeletal, p-tagged-hGBP-1 was immunoprecipitated from Jurkat T-cells after spreading on OKT3 mAb-coated slides and the proteins that co-immunoprecipitated with hGBP-1 were size fractionated on SDS-PAGE and identified by LTQ Orbitrap mass spectrometry (203). The ten possible interacting proteins of hGBP-1 in Jurkat T-cells were actin, tubulin B-polypeptide (TUBB), plastin-2, \(\beta\)-II-spectrin, stomatin-like protein (STOML2) and myosin regulatory light chain MRCL3 variant (MYLC2B), isoleucyl-tRNA synthetase (IARS), Alpha-enolase (ENO 1), bifunctional aminoacyl-tRNA synthetase (EPRS), Nuclease-sensitive element-binding protein 1 (YBX1), and Heat shock protein 90-alpha (HSP90A) (Table 1) (203). Only the role of \(\beta\)-II-spectrin and
plastin-2 were studied further. β-II-spectrin, which is a cytoskeletal protein, was proposed to enhance the expressions of antigen-presenting CD3 and CD45 receptors on T-cells (204, 205). To determine if hGBP-1 regulates the function of β-II-spectrin, the expression levels of CD3 and CD45 were analyzed by flow cytometry in the presence and absence of hGBP-1 in Jurkat T cells (203). Knockdown of hGBP-1 in Jurkat T cells resulted in higher expressions of CD3 and CD45 as compared to hGBP-1-expressing T-cells. This suggests that hGBP-1 is involved in regulation of β-II-spectrin-mediated cytoskeletal remodeling for the formation of antigen-presenting T-cell receptors (TCRs).

Because antigen-presenting TCRs are a prerequisite for T-cell activation, this predicts that hGBP-1 may interfere with TCR signaling (206). During, TCR signaling, plastin-2 is phosphorylated exclusively at serine 5 (207-210). This phosphorylation results in actin bundling and the expression of CD69 receptors on T-cells. To determine whether the interaction of hGBP-1 with plastin-2 interferes with TCR signaling, quantitative differences in the phosphorylation status of plastin-2 between hGBP-1-expressing and -knockdown T-cells were examined using SILAC-based sequential peptide enrichment and tandem mass spectrometry (203). The phosphorylation of plastin-2 at serine 5 in hGBP-1-expressing T-cells was reduced compared to hGBP-1-knockdown T-cells. This suggests that hGBP-1 inhibits plastin-2 phosphorylation during TCR signaling. To determine if hGBP-1 inhibits plastin-2-mediated expression of antigen-presenting CD69 receptors on T-cells, the expression of CD69 receptors on hGBP-1-expressing and -knockdown cells was analyzed by flow cytometry. hGBP-1-expressing T-cells showed reduced expression of CD69 as compared to hGBP-1 knockout T-cells (203). This confirms that hGBP-1 inhibits plastin-2-mediated TCR signaling. Altogether the data
suggest that hGBP-1 may regulate cytoskeleton-dependent TCR signaling. Another cytoskeletal protein, TUBB, was identified as a possible interacting protein of hGBP-1 in both HeLa and Jurkat T cells (Table 1) (201, 203). Also TUBB3, an isoform of TUBB, which is expressed in certain OVCAR3 ovarian cancer cell lines, interacts with hGBP-1 in vitro. Whether this interaction is real and is involved in ovarian tumor resistance to chemotherapy is still not clear (200).

To identify kinases that might interact with hGBP-1 in drug resistant ovarian carcinoma cells, purified hGBP-1-tagged V5-epitope was incubated with human proteins in high-density commercial protein arrays and the interacting proteins were detected with FITC-conjugated anti-V5 antibody (200). Sixteen kinases were identified which may interact with hGBP-1 (200). These are listed in the Table 1, as item 6. Only PIM1, a proto-oncogene, was studied further. The expressions of both hGBP-1 and PIM1 were up-regulated in A2780 ovarian cancer cell lines that were resistant to chemotherapeutic drugs including paclitaxel, cisplatin, and doxorubicin (200). However, it is still unknown if the interaction of hGBP-1 with PIM1 is real or if it modulates the cytoskeleton to mediate resistance to chemotherapy in ovarian tumor patients.

GBPs interacting partners during microbial and viral infections are listed in Table 1 (88, 100). For example, the interactions of mGBPs with proteins that are involved in autophagy, such as ATG4 and p62, and involved in oxidative killing, such as NADPH oxidase complex, inhibit the growth of Listeria monocytogenes (Lm) and Mycobacteria bovis (Mb) (Table 1) (100). Also, the interaction of hGBP-1 with the NS1 protein of Influenza A virus stops viral replication (Table 1) (88). In contrast, the interaction of hGBP-1 with the NS5 protein of Hepatitis C virus suppresses the GTPase activity of
hGBP-1, allowing viral growth (Table 1) (87). Altogether the data suggest that GBPs are involved in host defense upon infection with microbes and viruses.
Chapter Two
Results and Discussion

A. Effects of hGBP-1 on cell proliferation

The combined application of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) promotes the proliferation of both human microvascular umbilical vein endothelial cells (HUVECs) and microvascular endothelial cells (MVECs) (63). IFN-γ inhibits bFGF- and VEGF-induced proliferation of HUVECs and MVECs and induces the expression of hGBP-1. Forced expression of hGBP-1 alone inhibits bFGF- and VEGF-induced proliferation of HUVECs. Inhibition of proliferation of HUVECs was abolished when the IFN-γ-induced expression of hGBP-1 is inhibited by transduction with an hGBP-1-specific antisense cDNA construct. Together these data show that hGBP-1 inhibits the growth factor-induced proliferation of endothelial cells. Although IFN-γ treatment inhibits the proliferation of endothelial cells, it acts as a mitogen for human fibroblasts (211). IFN-γ also promotes the proliferation of murine NIH 3T3 cells and induces the expression of mGBP-2 (65). IFN-β also induces mGBP-2 expression but it does not influence the proliferation of NIH 3T3 cells. The level of mGBP-2 expression induced by IFN-β is lower than that induced by IFN-γ treatment. Forced expression of mGBP-2 to levels comparable to the level of mGBP-2 induced by IFN-γ enhanced the proliferation of NIH 3T3 cells. Alternatively, when ectopic mGBP-2 is expressed at a level comparable to the level of mGBP-2 induced by IFN-β does not influence fibroblast proliferation. Together these data suggest that a minimal threshold level of mGBP-2 may be required to promote fibroblast proliferation. Because hGBP-1 shows a concentration dependent increase in GTPase activity, this predicts that a
threshold level of GTPase activity of mGBP-2 may be required to promote fibroblast proliferation (25, 27). To determine whether hGBP-1 can alter the proliferation of various carcinoma cell lines, FLAG epitope-tagged hGBP-1 was ectopically expressed in MCF-7 breast adenocarcinoma cells, 2fTGH human sarcoma cells, and DU145 prostate carcinoma cells by transient transfection. Cell proliferation in the presence and absence of hGBP-1 was measured by BrDU incorporation. The expression of hGBP-1 in MCF-7 cells reduced the proliferating cells by approximately 40% (Figure 5). Similarly, the expression of hGBP-1 in 2fTGH cells reduced the proliferating cells by approximately 60% (Figure 5). However, the expression hGBP-1 in DU145 cells does not influence their proliferation (Figure 5). Another cell line in which hGBP-1 did not influence cell proliferation is TS/A breast adenocarcinoma (212). Thus hGBP-1 does not universally alter cell proliferation. The reason why hGBP-1 did not alter the proliferation of DU145 cells is not clear but possibly the level of hGBP-1 expression was below the required threshold level (65).

Only a sub-population of cells express the transfected proteins after transient transfection. To confirm that hGBP-1 inhibits proliferation, MCF-7 cells stably expressing tetracycline-regulatable hGBP-1 were generated by transfecting MCF-7 Tet-off cells with either pTRE-hygro2-myc-hGBP-1 or pTRE-hygro2 and then selecting in hygromycin. Colonies were isolated and expanded to generate clones. Individual clones were plated in the presence and absence of tetracycline for 24 hours and analyzed for hGBP-1 expression by immunoblot. All control clones containing empty vector do not express hGBP-1 either in the presence or absence of tetracycline (not shown). Two
Figure 5. hGBP-1 can inhibit cell proliferation. MCF-7, 2fTGH, and DU145 cells were co-transfected with either pCMV3-Flag-hGBP-1 and pCMV-β-galactosidase or pCMV3-Flag and pCMV-β-galactosidase. After 48 hours, control clones and hGBP-1-expressing cells were incubated with 25 μM BrDU for 3-4 hours. Cells were fixed, and incubated with anti-BrDU mouse monoclonal IgG1 and anti-β-galactosidase mouse monoclonal IgG2a, followed by incubation with Alexa Fluor 488-conjugated goat anti-mouse IgG1 isotype-specific secondary antibody and Alexa Fluor 594-conjugated goat anti–mouse IgG2a isotype-specific antibody. The results were expressed as average percentage of β-galactosidase positive cells that were BrDU positive ± SD (n = 3; * = p<0.05 compared to control cells). Black bars represent control cells and white bars represent hGBP-1-expressing cells. Figure by Jill Trendal, included with permission of Dr. Deborah J. Vestal.
hGBP-1-expressing clones (4-4 and 7-7) with relatively low basal levels and high inducible levels of hGBP-1 were chosen for further study. To determine if clones 4-4 and 7-7 express hGBP-1 at physiological levels, the amounts of hGBP-1 in both clones were compared to the amount of hGBP-1 induced by IFN-γ in MCF-7 cells after 24 hours of treatment. The level of hGBP-1 in clone 4-4 was higher than the level of hGBP-1 after IFN-γ treatment while the level of hGBP-1 in clone 7-7 was comparable to hGBP-1 induced by IFN-γ (Figure 6). To determine if hGBP-1 reduces the proliferation of MCF-7 cells, hGBP-1-expressing clones and control clones were plated in the presence or absence of tetracycline for 24 hours and their cell proliferation was measured. As expected, proliferation of both control clones (9-2c and 8-2c) was comparable either in the presence or in the absence of tetracycline (Table 2). However, hGBP-1 reduced the proliferation of clone 4-4 and 7-7 as compared to proliferation of 9-2c either in the presence or in the absence of tetracycline (Table 2). This indicates that hGBP-1 inhibits the proliferation of MCF-7 cells.

hGBP-1 is induced by either type I interferons (IFN-α/β) or type II interferon (IFN-γ) in a variety of cell types (63, 127, 213-215). Interferons also influence the proliferation of a variety of cell types (216-218). To determine whether IFN-γ and IFN-α2a inhibit the proliferation of MCF-7, 2fTGH, and DU145 cells, the cells were incubated with or without 500 U/ml IFN-γ or IFN-α2a for 24 hours and cell proliferation was measured. Neither IFN-γ nor IFN-α2a altered the proliferation of MCF-7 and 2fTGH cells (Table 3). However IFN-α2a reduced the proliferation of DU145 cells while IFN-γ did not (Table 3). Together these data indicate that interferons may influence the proliferation of certain cell lines. For example IFN-α inhibits the proliferation of MCC-1.
Figure 6. Characterization of MCF-7 cell lines with tetracycline-regulatable hGBP-1 expression. The MCF-7 pTet-off cell line was transfected with either pTRE-hygro2-myc-hGBP-1 or pTRE-hygro2-myc. After transfection, the cells were plated in multiple 10-cm dishes in media containing hygromycin and this medium was replaced every 4th day for 2 - 4 weeks. Individual colonies were isolated and expanded. Individual clones were plated in the presence and absence of tetracycline for 24 hours and analyzed for hGBP-1 expression by immunoblot. Two clones (4-4 and 7-7) with relatively low basal levels and high inducible levels of hGBP-1 were chosen for further study. To determine if clones 4-4 and 7-7 express hGBP-1 at physiological levels in the absence of tetracycline, the amounts of hGBP-1 in both clones were compared to the amount of hGBP-1 induced by 24 hours of IFN-γ treatment in MCF-7 cells. The clones, 4-4 and 7-7, were plated in the absence of tetracycline for 24 hours and 48 hours. MCF-7 cells were treated with 500 U/ml IFN-γ for 24 hours. Cell lysates (20 μg) were size fractionated on 8% SDS-PAGE and analyzed by immunoblotting with polyclonal anti-hGBP-1 and anti-actin antibodies. The positions of hGBP-1 and actin are indicated. Figure by Jill Trendal, included with permission of Dr. Deborah J. Vestal.
Table 1. hGBP-1 inhibits MCF-7 cell proliferation. Control MCF-7 clones (9-2c and 8-2c) and hGBP-1-expressing MCF-7 clones (4-4 and 7-7) were plated at 1 X 10^5 cells per well onto coverslips in 6-well dishes in the presence or absence of tetracycline for 24 hours. Cells were incubated with 0.125 mM BrdU for 2 hours, fixed, followed by incubation with anti-BrdU antibody and Alexa 488-conjugated goat anti-mouse IgG. Atleast 200 cells per coverslip were counted. The results were expressed as mean percentage of BrDU positive cells ± SD (n = 3; * = p<0.05 compared to 9-2C control cells with the same culture conditions). Table by Aaron Tipton, included with permission of Dr. Deborah J. Vestal.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>- tet</th>
<th>+ tet</th>
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<tr>
<td>9-2c</td>
<td>41.5 ± 1.1%</td>
<td>45.4 ± 2.3%</td>
</tr>
<tr>
<td>8-2c</td>
<td>38.8 ± 3.3%</td>
<td>43.0 ± 3.5%</td>
</tr>
<tr>
<td>7-7</td>
<td>29.7 ± 2.0% **</td>
<td>35.2 ± 1.2% **</td>
</tr>
<tr>
<td>4-4</td>
<td>25.2 ± 0.3% **</td>
<td>34.9 ± 0.2% **</td>
</tr>
</tbody>
</table>
Table 3

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Treatment</th>
<th>% BrDU positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>untreated</td>
<td>30.9 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>IFN-α2a</td>
<td>30.7 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>28.8 ± 2.0</td>
</tr>
<tr>
<td>DU145</td>
<td>untreated</td>
<td>35.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>IFN-α2a</td>
<td>27.4 ± 3.0 *</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>35.1 ± 1.8</td>
</tr>
<tr>
<td>2fTGH</td>
<td>untreated</td>
<td>33.9 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>IFN-α2a</td>
<td>31.8 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>34.0 ± 5.0</td>
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Table 2. Effects of interferon treatment on cell proliferation. MCF-7, 2fTGH, and DU145 cells were plated at 1 X 10^5 cells per well onto coverslips in 6-well dishes for 24 hours and treated with 500 U/ml IFN-γ or 1000 U/ml IFN-α2a for 3 days. After incubation with 25 μM BrDU for 3-4 hours, cells were fixed and incubated with anti-BrDU and Alexa 488-conjugated goat anti-mouse IgG. The results were expressed as average percentage BrDU positive cells ± SD (n = 3; * = p<0.05 compared to 9-2C in same culture conditions). Table by Jill Trendal, included with permission of Dr. Deborah J. Vestal.
skin carcinoma cells whereas IFN-γ inhibits the proliferation of MEC mammary carcinoma cells (216, 219). In contrast, proliferation of other mammary carcinoma cell lines (T47D, BT549, and MDA-MB-468) is not influenced by IFN-γ treatment (216). Interestingly, hGBP-1 is induced after IFN-γ treatment in MEC only and not induced in T47D, BT549, and MDA-MB-468 cells. Therefore it is possible that interferon signaling mediated through hGBP-1 may influence cell proliferation.

B. hGBP-1 identified as potential candidate for involvement in resistance to paclitaxel

Drug resistance is sometimes associated with the up-regulation and down-regulation of hundreds of genes (220, 221). To determine which genes were differentially regulated between paclitaxel-resistant and paclitaxel-sensitive cells, fold-changes in gene expressions were examined in SKOV-3 and OVCAR8 ovarian cancer and MCF-7 breast cancer cell lines. All three cell lines were made resistant to paclitaxel by continuous treatment with increasing concentrations of paclitaxel for 8 months (199). RNAs were isolated from the resistant and sensitive cells and analyzed by Affymetrix gene microarray. Fold changes of mRNA in resistant cells were determined as described in the legend of Table 4. In each of the paclitaxel-resistant cell lines the expressions of at least fifty genes were altered as compared to the paclitaxel-sensitive cell lines. However among those genes whose expression was altered, only eleven genes were common to all three paclitaxel resistant cell lines. Expressions of 8 of the 11 genes were up-regulated and 3 genes were down-regulated (Table 4). MDR1 was one of the up-regulated genes (Table 4). Because elevation of MDR1 was observed in ovarian carcinoma cells that
Table 3. List of up-regulated genes in paclitaxel-resistant cells. This table is modified from Duan et al. 2005. mRNAs were isolated from paclitaxel resistant and sensitive SKOV-3, OVCAR8, and MCF-7 cells and then reverse transcribed to single stranded cDNAs, followed by converting to double stranded cDNAs. In vitro transcription was performed in the presence of biotinylated UTP and CTP to produce biotin labeled cRNAs from double stranded cDNAs. cRNAs were then fragmented and hybridized to immobilized 23-oligomers probes in Affymetrix genechip HG-U95Av2 array. Subsequently un-hybridized cRNAs were removed by washing. After washing,
bound-cRNAs were incubated with phycoerythrin-labeled-streptavidin and then signal intensities were amplified by incubating with biotin-labeled anti-streptavidin antibody, followed by re-incubation with phycoerythrin-labeled-streptavidin. The signal intensities were measured with laser scanner. The signal intensities of single base mismatch oligo-probes were subtracted from the intensities from perfect match oligo-probes to determine the specific intensity of each probe set which represents the expression level of the mRNA of each gene. The fold changes of mRNA expression levels were determined by dividing the mRNA expression level of each gene in paclitaxel-resistance cells by the mRNA expression level of same the gene in paclitaxel-sensitive cells. Two-fold or greater change in mRNA expression level combined with a Mann-Whitney associated p-value less than 0.05 was considered significantly up-regulated gene expression. The expressions of up-regulated and down-regulated genes common in all three paclitaxel cell lines were listed in the table. Five of the eight up-regulated genes in paclitaxel-resistant cells were confirmed with RT-qPCR and/or Northern blot and in the table these genes are labeled with superscript a.
acquired resistance to paclitaxel (222, 223), this confirms the reliability of the gene array methodology used. Another gene whose expression was up-regulated in the paclitaxel-resistant cell lines was MGC4175, also called MM-TRAG (MDR1 and mitochondrial taxol associated gene) (Table 4). Expression of ectopic MM-TRAG in paclitaxel-sensitive SKOV-3 and OVCAR8 cells did not make them resistant to paclitaxel (222). This suggests that MM-TRAG is not sufficient for paclitaxel resistance. Of the remaining genes elevated in the paclitaxel-resistant cell lines, NF-κB-2, MGC14472, CATP-III, toll like receptor 6, and human testis specific basic protein genes have no experimental evidence that addresses whether these genes are involved in paclitaxel-resistance (Table 4). hGBP-1 was also up-regulated in paclitaxel-resistant cell lines (Table 4). To determine whether hGBP-1 is involved in paclitaxel resistance, hGBP-1 was stably expressed in paclitaxel-sensitive OVCAR-8 cells (198). hGBP-1-expressing cells and control transfectants were incubated in different concentrations of paclitaxel for 7 days and fold-changes in paclitaxel resistance were measured. The fold change in paclitaxel resistance was calculated by dividing the IC50 of paclitaxel-resistant cells by the IC50 of paclitaxel-sensitive cells. hGBP-1 decreased paclitaxel sensitivity by approximately 6-7 fold, suggesting that hGBP-1 contributes to paclitaxel resistance.

MCF-7 and T47D breast cancer cells acquire morphologically identifiable apoptotic cells after paclitaxel treatment (224). This suggests that paclitaxel induces apoptosis. To determine whether hGBP-1 inhibits paclitaxel-induced apoptosis, SKOV-3 cells were transiently co-transfected with FLAG epitope-tagged hGBP-1 and β-galactosidase. hGBP-1-expressing cells and control cells were treated with 5μM paclitaxel for 24 hours and the cells were analyzed for apoptosis. hGBP-1 reduced the
numbers of TUNEL positive cells by approximately 78%, indicating that hGBP-1 can inhibit paclitaxel-induced apoptosis (Figure 7). To confirm that hGBP-1 protects cells from paclitaxel-induced killing, MCF-7 cells with tet-regulated hGBP-1 were plated in the absence of tetracycline for 24 hours and then treated with paclitaxel for 24 hours. hGBP-1 reduced paclitaxel-induced cell death by approximately 67% (Figure 8). Thus hGBP-1 protects MCF-7 from paclitaxel-induced cell death.

C. mGBP-2 inhibits paclitaxel-induced apoptosis

To determine whether mGBP-2 confers paclitaxel resistance, mGBP-2-expressing and control NIH 3T3 fibroblasts were treated with 5 μM paclitaxel for 12 and 24 hours and the numbers of dead cells were determined by trypan blue exclusion (225). The numbers of dead cells in paclitaxel treated samples were normalized by subtracting the numbers of trypan blue positive cells in DMSO (vehicle) treated samples from the numbers of trypan blue positive cells in paclitaxel treated samples (225). mGBP-2 reduced cell death by approximately 30-50%.

To determine the regions of mGBP-2 essential for the inhibition of paclitaxel-induced cell death, five mutants of mGBP-2 were generated. The biochemical properties of GBPs have been best studied with hGBP-1. Because mGBP-2 is predicted to have a similar structural to hGBP-1, the mGBP-2 mutants were based on previously characterized hGBP-1 mutations (15, 225). hGBP-1 is composed of an N-terminal nucleotide-binding LG domain, followed by a short-intermediate region and then a C-terminal α-helical domain (Figure 1) (25). hGBP-1 can exist as monomers, dimers, and
Figure 7. **hGBP-1 inhibits paclitaxel-induced apoptosis of SKOV-3 cells.** A) SKOV-3 cells were plated at 6 X 10^4 cells per well onto coverslips in 6-well dishes and 24 hours later cells were co-transfected with either pCMV2-Flag and pCMV-β galactosidase or pCMV2-Flag-hGBP-1 and pCMV-β galactosidase. After another 24 hours, cells were treated with either 5 μM paclitaxel or DMSO for 18 hours. Cells were then fixed and processed for TUNEL staining (Dead End™ Flurometric TUNEL system, promega), followed by incubation with mouse anti-β-galactosidase and Alexa-594-conjugated anti-mouse Ig. The results were expressed as average percentage of β-galactosidase positive cells that were TUNNEL positive ± SD (n = 3; * = p<0.01 compared to paclitaxel treated control cells). White bars represent cells treated with DMSO and black bars represent cells treated with paclitaxel. Figure by Shadia Nada, included with permission of Dr. Deborah J. Vestal.
Figure 8. hGBP-1 reduced paclitaxel-induced cell death in MCF-7 cells.  

A) hGBP-1-expressing cells and control transfectants were plated at 2 x 10^4 cells in the absence of tetracycline. After 24 hours, cells were treated with either 5 μM paclitaxel or DMSO for 24 hours. The numbers of dead cells were measured by trypan blue exclusion. The results were expressed as average percentage of trypan blue cells ± SD (n = 3; * = p<0.05 compared to paclitaxel-treated control cells). White bars represent cells treated with DMSO and black bars represent cells treated with paclitaxel. 

B) At the end of the assay hGBP-1 expression was analyzed by Immunobloting. Figure by Shadia Nada, included with permission of Dr. Deborah J. Vestal.
tetramers in living cells (27). C290 mGBP-2 is a truncated version of mGBP-2 containing helices α6 through α13 (amino acids 290 to 589) (225, 226). Because it is not certain which region of the C-terminal helix of mGBP-2 interacts with downstream targets, a second truncated version of mGBP-2 was generated which contains only the α12 and α13 helices and was designated C473 mGBP-2 (225). D184N mGBP-2 contains a single point mutation in the LG domain at position 184 which replaces lysine with asparagine (225). D184 hGBP-1 exhibits reduced binding affinity for GTP, GDP, and GMP under physiological conditions (25, 32). But D184N mGBP-2 is still expected to hydrolyze GTP and may form dimers under physiological conditions. S52N mGBP-2 contains a single point mutation in the LG domain at position 52, in which serine was replaced with asparagine (225). Based on S52N hGBP-1, S52N mGBP-2 is expected to lack GTPase activity and remain as a nucleotide-free monomer (32). The extreme C-terminal region of mGBP-2 (amino acids 586 to 589) has a CaaX-motif CTIL (cysteine, threonine, isoleucine and leucine) (226). STIL mGBP-2 has a single amino acid substitution at position 586 which replaces cysteine with serine (225). To determine the effect of the mGBP-2 mutants on paclitaxel-induced apoptosis, the five FLAG-epitope tagged mGBP-2 mutants were co-transfected individually with β-galactosidase into NIH 3T3 cells and the cells were examined for apoptosis after paclitaxel treatment (225). The TUNEL positive cells in samples treated with paclitaxel were normalized by subtracting the TUNEL positive cells in DMSO-treated samples from the TUNEL positive cells in paclitaxel treated samples (225). C473 mGBP-2, C290 mGBP-2, S52N mGBP-2, D184N mGBP-2, and wild-type mGBP-2 (mGBP-2 WT) inhibited paclitaxel-induced apoptosis. This suggests that either structural changes in C-terminal helices or GTP-hydrolysis is not
required to inhibit paclitaxel-induced apoptosis. However, STIL mGBP-2 did not inhibit paclitaxel-induce apoptosis. This indicates lipid attachment to mGBP-2 is required for protection from paclitaxel-induced apoptosis.

**D. hGBP-1 is not directly induced by paclitaxel**

To determine whether the up-regulation of hGBP-1 in paclitaxel resistant cells is because paclitaxel induces the expression of hGBP-1, SKOV-3 cells were treated with 5 μM paclitaxel for 24 and 48 hours. In addition, SKOV-3 cells were treated with either IFN-γ or IFN-α2a for 24 and 48 hours (Figure 9). As expected, hGBP-1 was robustly induced by IFN-γ treatment for 24 and 48 hours and was induced at low level by IFN-α2a for 24 hours (Figure 9). However paclitaxel did not induce hGBP-1 during 48 hours of treatment (Figure 9). This suggests that short-exposure to paclitaxel does not induce hGBP-1 expression in carcinomas. Thus one can ask the question whether cytokine-signaling due to adaptive immune responses in the tumor areas induces the expression of hGBP-1 and promotes resistance to paclitaxel. A study on colorectal carcinoma (CRC) shows that the presence of IFN-γ-dominated T-helper type I (Th1) cells in tumors may lead to induction of IFN-target genes in the tumor (227-229).

**E. IFN-γ protects ovarian carcinoma cell lines from paclitaxel-induced apoptosis**

IFN-αA treatment inhibits staurosporine-induced apoptosis of FHs74 intestinal epithelial cells and induces the expression of hGBP-1 (127). Knockdown of IFN-αA
Figure 9. Paclitaxel does not induce hGBP-1 expression in SKOV-3 cells within 48 hours. SKOV-3 cells were plated at 2.4 X 10^5 cells in 6-cm dishes and after 24 hours cells were treated with 5 μM paclitaxel for 24 and 48 hours. SKOV-3 cells were plated for IFN treatment at 1.2 x 10^5 cells in 6-cm dishes and after 24 hours, cells were incubated with either 500 U/ml IFN-γ or 1000 U/ml IFN-α2a for 24 and 48 hours. Cell lysates (20 μg) were size fractionated on 8% SDS-PAGE and analyzed by immunoblot with polyclonal anti-hGBP-1 and anti-actin antisera. LE represents longer exposure time for detection of low level of hGBP-1 induction with IFN-α2a. The positions of hGBP-1 and actin are indicated. Figure by Shadia Nada, included with permission of Dr. Deborah J. Vestal.
induced hGBP-1 in FHs74 cells abolishes the IFN-mediated inhibition of staurosporine-induced apoptosis. This suggests hGBP-1 inhibits staurosporine-induced apoptosis of FHs74. In addition, knockdown of hGBP-1 in IFN-γ-treated SK-CO15 intestinal epithelia cells enhances TNF-α-induced apoptosis (128). To determine whether interferon treatment inhibits paclitaxel-induced apoptosis, SKOV-3 cells were pre-treated with either IFN-γ or IFN-α2a for 24 hours and then treated with 5 μM paclitaxel for 18 hours and examined for apoptosis. IFN-γ reduced paclitaxel-induced TUNEL positive SKOV-3 cells by approximately 67% (Figure 10). However IFN-α2a did not influence the apoptosis of SKOV3 cells after paclitaxel treatment. This suggests that IFN-γ inhibits paclitaxel-induced apoptosis.

F. hGBP-1 is up-regulated in a subset of recurrent ovarian tumors from patients treated with paclitaxel

To investigate whether hGBP-1 is up-regulated in ovarian tumors, tumor tissues from ovarian cancer patients were collected and examined for hGBP-1 mRNA expression. The tumor samples collected from the initial diagnostic/debulking surgeries prior to chemotherapy are referred to as new tumors. Those tumors that were collected during debulking surgeries following recurrence of the cancer after chemotherapy are referred to as recurrent tumors. To determine the normal variation in hGBP-1 expression in benign ovaries, five benign ovaries (N1 through N5) were obtained from the Midwestern Division of the Cooperative Human Tissue Network. Total RNA was isolated from samples and used to analyze levels of hGBP-1 and GAPDH mRNA by real time RT-PCR. In each tissue sample the hGBP-1 mRNA level was normalized to the
Figure 10.  IFN-γ inhibits paclitaxel-induced apoptosis of SKOV-3 cells.  SKOV-3 cells are plated at 6 X 10⁴ cells per well onto coverslips in 6-well dishes and 24 hours later cells were treatment with either 500 U/ml of IFN-γ or 1000 U/ml IFN-α2a for 26 hours. During the last 18 hours of IFN treatment, 5 μM paclitaxel or DMSO was added. The cells were then fixed and processed for TUNEL staining (Dead End™ Fluometric TUNEL system, Promega). The results were expressed as average percentage of TUNEL positive cells ± SD (n = 3; * = p<0.05 compared to paclitaxel treated control). White bars represent cells treated with DMSO and black bars represent cells treated with paclitaxel. Figure by Shadia Nada, included with permission of Dr. Deborah J. Vestal.
level of GAPDH mRNA. Fold-changes of hGBP-1 in samples were determined by dividing the hGBP-1 mRNA level after normalization in each sample by the hGBP-1 mRNA level in benign sample 41. In benign ovaries the levels of hGBP-1 mRNA vary between 0.7- to 1.3-fold and cluster around the level of hGBP-1 mRNA in benign tumor sample 41 (Figure 11). Levels of hGBP-1 mRNA equal to or greater than 2-fold higher than those of sample 41 were considered significantly up-regulated. Of the eighteen new tumors, thirteen (72%) had hGBP-1 mRNA levels either equal to or lower than hGBP-1 in normal ovaries and only three (17%) had elevated hGBP-1 mRNA (Figure 11A). Thus hGBP-1 was not up-regulated in the majority of ovarian tumors. To determine whether the levels of hGBP-1 mRNA were elevated in recurrent ovarian tumors after patients had treatment that included a taxane, ten recurrent tumors from ovarian cancer patients were collected and examined for levels of hGBP-1 mRNA. Eight of the ten (80%) samples were from patients who had been treated with a chemotherapy regime that included paclitaxel or docetaxel (taxotere). Of the remaining two samples, one (sample 12) was from a patient who had not received a taxane treatment and one (sample 37) was from a patient whose treatment was unknown. The level of hGBP-1 mRNA was elevated in 4 of the 10 (40%) samples of recurrent tumors (Figure 11B). All 4 samples with elevated hGBP-1 were from patients who had been treated with a taxane. Thus hGBP-1 was elevated in a higher percentage of recurrent tumors than in new tumors. Of the 8 recurrent tumors that were examined from patients after taxane treatment 4 (50%) had elevated hGBP-1. This suggests that up-regulation of hGBP-1 in recurrent ovarian tumors could be a predictive biomarker for resistance to treatment with a taxane.
Figure 11

A

Fold difference (hGBP-1)

Benign
Tumor

41 N1 N2 N3 N4 N5 2 13 18 19 20 35 42 43 44 46 47 48 49 57 59 66 73 77

B

Fold difference (hGBP-1)

Benign
no taxane
paclitaxel
taxotere
unknown

41 N1 N2 N3 N4 N5 5 12 25 30 31 32 34 37 38 69
Figure 11. hGBP-1 is upregulated in recurrent tumors of ovarian cancer patients.

Tumor samples are collected from ovarian cancer patients prior to initiation of chemotherapy are referred to as new tumors. Those tumors that are collected during debulking surgeries following recurrence of the cancer after chemotherapy are referred to as recurrent tumors. Five benign ovaries (N1 through N5) were obtained from the Midwestern Division of the Cooperative Human Tissue Network. Total RNAs were extracted from flash frozen tumor and normal tissue samples. mRNAs were purified from total RNAs and reverse transcribed to generate cDNAs for analysis of hGBP-1 and GAPDH levels by real time RT-PCR (TaqMan, Life Technologies). In each tissue sample hGBP-1 mRNA level was normalized to GAPDH mRNA level. Benign tumor sample 41 was run on each plate as a reference sample for hGBP-1 mRNA and set to 1. Fold-changes of hGBP-1 mRNA were calculated by dividing the hGBP-1 mRNA level in each sample by the hGBP-1 mRNA level in benign sample 41. Fold-difference of hGBP-1 in benign ovaries N1 to N5 range were 0.7- to 1.3-fold and clustered around hGBP-1 in benign sample 41. Levels of hGBP-1 equal to or greater than 2-fold higher than those of sample 41 were considered significantly up-regulated. Results were expressed as fold-change of hGBP-1 level ± S.D (n = 2 in triplicate). A) Graphical representation of hGBP-1 levels in new tumors. Three of eighteen (17%) samples had elevated expression of hGBP-1. B) Graphical representation of hGBP-1 levels in recurrent tumors. Four of ten (40%) samples (sample number 5, 32, 34, and 69) had elevated expression of hGBP-1. Figure by Suzan Wadi, included with permission of Dr. Deborah J. Vestal.
G. TUBB3 mRNA is elevated in human ovarian tumors

(i) TUBB3 was elevated in paclitaxel-resistant A2780 ovarian carcinoma cells

TUBB3, an isotype of β-tubulin, is up-regulated in some paclitaxel-resistant A2780 ovarian carcinoma and MCF-7 breast carcinoma cells (230, 231). TUBB3 is able to induce microtubule instability and counteract the microtubule-stabilizing drug, paclitaxel (192). Surprisingly, knockdown of TUBB3 in H460 non-small cell lung cancer not only abolished resistance to microtubule-targeting drugs such as paclitaxel but also abolished resistance to DNA-damaging drugs such as cisplatin and doxorubicin (232, 233). To determine whether the expression of TUBB3 is associated with the expression of hGBP-1 in drug resistant carcinomas cell lines, three A2780 ovarian carcinoma cell lines that are resistant to paclitaxel, cisplatin, and doxorubicin, and parental A2780 cell line were examined for the expression of TUBB3 and hGBP-1 mRNAs and proteins (234). The expression of TUBB3 was up-regulated in all three of the drug resistant A2780 cell lines as compared to parental A2780 cells. In contrast, the expression of hGBP-1 was down-regulated in all three of the drug resistant A2780 cell lines as compared to parental A2780 cells. This indicates that hGBP-1 expression is down-regulated in TUBB3-expressed ovarian carcinoma cells. Interestingly, the expression of hGBP-1 was also not up-regulated in another paclitaxel-resistant MCF-7-derived cell line (data not shown).

Having found that hGBP-1 is not expressed when TUBB3 is expressed in drug-resistant A2780 cells, investigators asked if hGBP-1 is present in the cytoskeleton of cells where it could interact with TUBB3 and possibly regulate its function. To determine
whether hGBP-1 interacts with TUBB3 in drug resistant cells, the cytoskeletal and soluble fractions of A2780 cisplatin-resistant cells and parental A2780 cells were examined for hGBP-1 (234). In both cell lines, hGBP-1 was found predominantly in the soluble fraction with low levels in the cytoskeletal fraction. Because hGBP-1 is reported to localize on vesicle-like structures that may run along the microtubules in living cells, this opens the possibility that the hGBP-1 in the cytoskeleton may be the consequence of hGBP-1 association with vesicles (14, 83). It may also be a consequence of the interaction of hGBP-1 with actin (201). Subcellular fractionation does not show a direct interaction of hGBP-1 with tubulin. To determine whether hGBP-1 can physically interact with TUBB3, far-western blots were performed using cell lysates of OVCAR3 cells expressing TUBB3 as bait and recombinant bacterially-expressed hGBP-1 as prey. Recombinant hGBP-1 and recombinant carbonic anhydrase (negative control) were size fractionated by SDS-PAGE and transferred to a membrane. The membrane was incubated with OVCAR3 cell lysates. For cross-linking the interacting proteins, the membrane was incubated with carbodiimide before washing and then incubated with anti-β III tubulin. A band was detected at 67 kDa that corresponds to the size of hGBP-1, suggesting that hGBP-1 could interact with TUBB3 (234). However whether in their native confirmations hGBP-1 and TUBB3 are able to interact with each other remains unclear. Also, whether hGBP-1 interacts with TUBB3 within cells needs to be investigated.

(ii) TUBB3 in human ovarian tumors

Because De Donato et al. proposed that hGBP-1 can interact with TUBB3 in carcinoma cells, it is important to determine if the expression of TUBB3 is associated
with the expression of hGBP-1 in ovarian tumors (200). To examine the TUBB3 level in ovarian tumors, tumor samples that were used to examine the levels of hGBP-1 mRNA were used to determine the levels of TUBB3 mRNA. The fold-change of TUBB3 mRNA in tumors and normal ovaries was determined as described (Figure 11). In benign ovaries (N1 through N5) the levels of TUBB3 mRNA varies between ~0.1- to 1.1-fold and cluster around the level of TUBB3 mRNA in benign tumor sample 41 (Figure 12). Levels of TUBB3 mRNA equal to or greater than 2-fold higher than sample 41 were considered up-regulated. In new tumors, TUBB3 mRNA was elevated in 9 of the 17 (53%) samples (Figure 12A and B). Of the 10 recurrent tumors, TUBB3 mRNA was elevated in 4 (40%) samples (Figure 12C). Therefore in this pilot study TUBB3 was elevated in fewer recurrent tumors than new tumors, suggesting that the expression of TUBB3 could up-regulated in ovarian carcinoma prior to chemotherapy.

(iii) hGBP-1 and TUBB3 are not always both expressed in human ovarian tumors

To determine the expression patterns of hGBP-1 and TUBB3 in ovarian tumors in ovarian tumors, both new and recurrent tumors were examined individually for levels of hGBP-1 and TUBB3 mRNA. Both hGBP-1 and TUBB3 mRNAs were up-regulated in 2 of the 17 (12%) new tumors (Figure 13A). Of the remaining new tumors, 8 (47%) samples had elevated TUBB3 mRNA only and 1 (6%) sample had elevated hGBP-1 mRNA only (Figure 13A). This suggests that the expression of hGBP-1 is not strictly associated with the expression of TUBB3 in new tumors. To examine the expression patterns of hGBP-1 and TUBB3 in recurrent ovarian tumors after patients were administered treatment that includes taxane, 10 recurrent tumors were examined. Two of the ten recurrent tumors were from patients who were treated either without taxane
Figure 12. **TUBB3 mRNA expression is upregulated in some ovarian cancer tumors.** The same samples in Figure 11 were used to determine TUBB3 mRNA levels. In each tissue sample TUBB3 mRNA level was normalized to GAPDH mRNA level. TUBB3 mRNA level in benign tumor sample 41 was set to 1. Fold-change of TUBB3 mRNA was calculated by dividing the TUBB3 mRNA level in each sample by the TUBB3 mRNA level in benign sample 41. Fold difference of TUBB3 in normal tissue samples N1 to N5 were from 0.1- to 1.1-fold which clustered around TUBB3 level in benign tumor sample 41. Levels of TUBB3 equal to or greater than 2-fold higher than those of sample 41 were considered significantly up-regulated. Results were expressed as fold-change of TUBB3 level ± S.D (n = 1 in triplicate). **A)** Graphical representation of TUBB3 levels in new tumors. Nine of eighteen (50%) samples had elevated TUBB3. **B)** In order to expand the Y-axis and allow for better view of TUBB3 levels in new tumors, the sample 73 was excluded. **C)** Graphical representation of TUBB3 levels in recurrent tumors. Four of ten (40%) samples (sample number 5, 30, 32 and 37) had elevated expression of TUBB3. Figure by Suzan Wadi, included with permission of Dr. Deborah J. Vestal.
Figure 13. Levels of both hGBP-1 and TUBB3 mRNAs in ovarian tumors. Representation of the levels of both hGBP-1 and TUBB3 mRNAs in each tumor sample from Figures 11 and 12 are shown. The X-axis represents levels of hGBP-1 mRNA and the Y-axis represents levels of TUBB3 mRNA. Black diamonds indicate those samples in which neither hGBP-1 nor TUBB3 mRNAs were up-regulated, blue diamonds indicate those samples in which both hGBP-1 and TUBB3 mRNAs were up-regulated, green diamonds indicate those samples in which only TUBB3 mRNA was up-regulated and red diamonds indicate those samples in which only hGBP-1 was up-regulated. A) Graphical representation of 17 new tumor samples with levels of both hGBP-1 and TUBB3 mRNAs. B) Graphical representation of 10 recurrent tumor samples with levels of both hGBP-1 and TUBB3 mRNAs. Figure by Dr. Deborah Vestal, included with permission of Dr. Deborah J. Vestal.
or whose treatment was unknown. Those two tumors did not have elevated hGBP-1 or TUBB3 mRNA (Figure 13B). Of the remaining samples, two (20%) tumors had elevation of both hGBP-1 and TUBB3 mRNAs. Two (20%) tumors had elevation of hGBP-1 mRNA only and another two (20%) tumors had elevation of TUBB3 mRNA only (Figure 13B). This suggests that the expressions of hGBP-1 and TUBB3 are not always inversely associated in ovarian tumors which become resistant to treatments that include taxane.

H. Expression of hGBP-1 and docetaxel-resistance

hGBP-1 is up-regulated in some paclitaxel-resistant ovarian and breast cancer cell lines (199). Forced expression of hGBP-1 in paclitaxel-sensitive OVCAR8 cells conferred moderate resistance to paclitaxel-induced cytotoxicity (198). Docetaxel has 1.8 times higher affinity for tubulin than paclitaxel but both drugs suppress microtubule dynamic instability (235, 236). Because both drugs target β-tubulin, paclitaxel and docetaxel resistance may be acquired through similar mechanisms. To determine whether hGBP-1 is involved in docetaxel-resistance, the level of hGBP-1 expression was examined in docetaxel-resistant and -sensitive prostate cancer cell lines (237). hGBP-1 was up-regulated in docetaxel-resistant DU145 cells but was down-regulated in docetaxel resistant PC-3 cells. When hGBP-1 was stably expressed in docetaxel resistant PC-3 cells, the numbers of viable cells were decreased with docetaxel treatment by 1.67-fold. Thus hGBP-1 is not consistently up-regulate in docetaxel-resistant cell lines and it remains unclear if hGBP-1 plays a role in docetaxel resistance.
Chapter Three

Conclusion

hGBP-1 is up-regulated in MCF-7 breast and SKOV-3 and OVCAR-8 ovarian cancer cell lines when they become resistant to paclitaxel (199). The forced expression of hGBP-1 in OVCAR-8 cells confers resistance to paclitaxel-induced cytotoxicity in vitro (198). hGBP-1 expression in ovarian and breast cancer cells provides protection against paclitaxel-induced apoptosis. A analyses of ovarian tumors shows that hGBP-1 is not elevated in primary tumors but it is up-regulated in recurrent tumors from patients who had received chemotherapy that included paclitaxel or docetaxel. Together these data suggest that hGBP-1 can serve as a diagnostic biomarker for the development of paclitaxel resistance in women with ovarian cancer. Thus identification of a new marker would prevent delays in switching new choices of drugs for the chemotherapy.
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