American Journal of Immunology

Original Research Paper

Regulation of Gene Expression by β-Glucans

[1]Mary O. Huff and [2]Carolyn M. Klinge

[1]Department of Biology, Bellarmine University, Louisville, KY 40205, USA
[2]Department of Biochemistry and Molecular Genetics,
University of Louisville School of Medicine, Louisville, KY 40292, USA

Article history
Received: 15-11-2016
Revised: 12-01-2017
Accepted: 29-03-2017

Corresponding Authors:
Mary O. Huff
Department of Biology,
Bellarmine University,
Louisville, KY 40205, USA
Tell: 502-272-8495
Email: mhuff@bellarmine.edu

Carolyn M. Klinge
Department of Biochemistry
and Molecular Genetics,
University of Louisville School
of Medicine, Louisville, KY
40292, USA
Tell: 502-852-3668
Email: Carolyn.klinge@louisville.edu

Abstract: β-D-glucans are diverse polysaccharides derived from plant cell walls, fungi and bacteria. β-D-glucans are composed of D-glucose monomers linked by (1-3) β-glycosidic bonds with varying amounts of (1-6) or (1-4) linked side chains that bind cell surface receptors ultimately triggering changes in intracellular pathways and gene transcription. β-D-glucans have anti-viral, immunomodulatory and anti-cancer activities. This article reviews the current identity and putative function of genes regulated by β-glucans purified from various sources in human cancer and immune cells and in murine dendritic, macrophage cells and lungs. β-D-glucans increase the expression of numerous cytokines and immunomodulatory factors and their receptors in dendritic cells. Pathways and transcription factors involved in the cellular responses to β-glucans are summarized.

Keywords: Glucan, Transcription, Cytokines

Introduction

β-D-glucans are diverse polysaccharides derived from plant cell walls, fungi and bacteria composed of D-glucose monomers linked by (1-3) β-glycosidic bonds and variable amounts of (1-6) and (1-4) branches. β-D-glucans are considered to be "biological response modifiers" because they exhibit antibacterial, antiviral, anti-coagulatory, anti-tumoral immunomodulatory and wound-healing activities (Bohn and BeMiller, 1995). In addition, β-D-glucans stimulate the immune system against infectious pathogens and cancer (Saraswat-Ohri *et al*., 2011; Chan *et al*., 2009; Aleem, 2013). When ingested in plant materials, β-glucans are absorbed in the small intestine, internalized and fragmented by macrophages and subsequently carried to the spleen, lymph nodes and bone marrow (Chan *et al*., 2009). Here it is believed that the soluble β-glucan fragments are released and taken up by circulating granulocytes, monocytes and dendritic cells while insoluble fragments induce a cellular response by binding to cell surface receptors. Several types of β-glucan receptors have been identified including several immune receptors, e.g., Dectin-1, Complement Receptor (CR3), CD11b/CD18, Mac-1, aMb integrin (Ross, 2000), Scavenger Receptors (SR), Lactosylceramide (LacCer) and toll-like receptors, e.g., TLR-2/6 and trigger responses in macrophages, neutrophils, monocytes, natural killer cells and dendritic cells *in vitro* (Chan *et al*., 2009; Kim *et al*., 2011; Legentil *et al*., 2015). β-glucans also bind to L-Ficolin (Legentil *et al*., 2015). Through this interaction with plasma membrane receptors, β-glucans potentiate intracellular signaling pathways that ultimately activate transcription factors such as NFκB resulting in both innate and adaptive immune responses (Li *et al*., 2010).

In addition to immunomodulatory activity, β-glucans have been reported to have direct anti-cancer activity *in vitro*. A water-soluble β-glucan extract from the mycelia of *Poriacocos* inhibited the viability (MTT assay) of MCF-7 human breast cancer cells with an IC_{50} of 400 μg mL^{-1} and decreased cyclin D1 and cyclin E protein expression (Zhang *et al*., 2006). We reported that a purified preparation of β-D-glucan (from barley) dissolved in DMSO - but not water- inhibited the growth of estrogen receptor α (ERα)+ MCF-7 cells with an IC_{50} of ~164±12 μg mL^{-1} compared to normal breast epithelial (ERα-) MCF-10A cells, IC_{50}~464 μg mL^{-1} (Jafaar *et al*., 2014). β-glucan inhibited the estradiol (E_2)-independent, tamoxifen (TAM) and fulvestrant-resistant, ERα + LCC9 and LY2 cells (derived from MCF-7 cells)

with IC_{50} values of 4.6±0.3 and 24.2±1.4 μg mL^{-1}, respectively (Jafaar *et al*., 2014). In contrast, the "triple negative/basal-like" MDA-MB-231 breast cancer cells were not growth inhibited by β-glucan (Jafaar *et al*., 2014). Conversely, aqueous-soluble β-glucans reportedly had no direct cytotoxic effects on a panel of common cancer cell lines including blastoma, carcinoma and sarcoma cells (Chan *et al*., 2009).

There are many studies of β-glucans purified from the cell walls of yeast, fungi and plants as immunomodulators that increase the levels of pro-inflammatory cytokines, chemokines and cell adhesion molecules (Novak and Vetvicka, 2008). We will review immunomodulatory genes regulated by β-glucans in human and mouse cells. In addition, we reviewed the literature to identify additional genes increased or inhibited by glucan treatment of cells *in vitro*. We summarize the downstream effects of these transcriptional changes and how they inform the mechanisms by which glucans act in various cell types.

Glucans Increase Gene Transcription in Human Cancer Cells and Immortalized Normal Cells

A number of reports have examined the effect of β-glucan on transcription in human and murine immune cells with a few studies examining the activity of glucans in human cancer cells. Table 1 summarizes those genes that were transcriptionally induced by β-glucan in the cells examined. First, we will review the results in human cells.

β-Glucan in Breast Cancer Cells

In our lab, it was shown that a purified β-D-glucan isolated from barley and dissolved in DMSO, but not water, inhibited cell viability in two human breast cancer cells lines: MCF-7 and LCC9, an endocrine-resistant (estradiol, tamoxifen and fulvestrant) cell line derived from MCF-7. Using two concentrations of β-D-glucan, we examined the expression of a set of genes implicated in breast cancer in both cell lines using PCR array analysis.

Table 1. Genes upregulated by glucan in cell systems. The concentration, form and source of β-glucan used in the experiment is indicated. The comments include information about the genes regulated taken from the references cited and the GeneCards human gene database http://www.genecards.org/ LMW = low molecular weight

Gene (s)	Cell system	β-glucan	Ref.	Comments
BIRC5, BRCA1, BRCA2, CCNA1, PGR, RASSF1	MCF-7 human breast cancer cells	10 or 50 μg mL^{-1} β-D glucan purified from barley and purchased from Sigma (cat. No. G6513, purity ~97%), dissolved in DMSO	(Jafaar *et al*., 2014)	BIRC5 is anti-apoptotic;BRCA1 and BRCA2 are involved in DNA repair, Cyclin A1(*CCNA1*) regulates cell cycle progression, progesterone receptor (*PGR*) is a breast cancer differentiation marker, RASSF1 (*NORE2A*) is a scaffolding protein that functions as a tumor suppressor
CTSD, PTGS2	MCF-7 human breast cancer cells	10 μg mL^{-1} β-D glucan	(Jafaar *et al*., 2014)	Cathepsin D (*CTSD*) is an acid protease; COX-2 (*PTGS2*) increases pro-inflammatory cytokines
MK167	MCF-7 human breast cancer cells	50 μg mL^{-1} β-D glucan	(Jafaar *et al*., 2014)	Ki-67 is a marker of cell proliferation
EGF, GLI1, HIC, IGF1, IGFBP3, PTGS2	LCC9 endocrine-resistant human breast cancer cells	10 or 50 μg mL^{-1} β-D glucan	(Jafaar *et al*., 2014)	GLI1 is a zinc finger transcription factor; HIC is the gene *MDFIC* is a transcriptional regulator
TP53, *CDKN1B*	MCF-7 human breast cancer cells	Fungal β-glucan (Botryosphaeran, BOT) was produced by *B. rhodina* MAMB-05 grown on nutrient medium containing glucose (BOT_{GLC}) and fructose (BOT_{FRU}). Cells were treated with 100 μg mL^{-1} for 48 h	(Queiroz *et al*., 2015)	P53 and P27 are involved in cell cycle arrest
CD86	Dectin-1(+)CR3(-) human Burkitt (B-cell) lymphoma cell lines (Daudi and Raji)	Aqueous underivatized β glucan from *Saccharomyces cerevisiae* (1 μg mL^{-1}) for Raji cells and 0.1 μg/ml for Daudi cells	(Harnack *et al*., 2011)	Maturation marker-measured cell surface expression by flow cytometry. Observed peak increase at 48 h tx. Both cell lines express dectin-1, the major β-glucan receptor.
HSP70 protein, *MUC1*	Human gastric carcinoma cells	LMW β-glucan purified from conditioned medium of *Aureobasidium pullulans* GM-NH-1A1 with ~ 70% β-(1-6) branches 100 μg mL^{-1}	(Tanaka *et al*., 2011)	MUC1 is mucin 1, a transmembrane protein.

Table 1. Continuous

PKC, CXCL8 (IL-8 gene), *CDC42, BBC3* (PUMA)	Detroit-573 human fibroblasts and Ha Cat human keratinocytes	6 different synthetic glucans-no concentrations or treatment times indicated	(Vetvicka *et al*., 2011)	Regulators of cell growth and PUMA is a BCL-2 pro-apoptotic protein
IL-10, IL-12, IL-23 transcripts	Human monocyte-derived Dendritic Cells (DCs) from healthy donors	zymosan (β-glucan from *Saccharomyces cerevisiae* (InvivoGen) 10 and 200 μg mL^{-1}	(Gerosa *et al*., 2008)	reviewed in (Lyakh *et al*., 2008)
*IL*23A, *PTGS2*	Human monocyte-derived DCs from healthy donors	β(1,3)-glucan Zymosan from *Saccharomyces cerevisiae* (Sigma) 1 mg mL^{-1},4 h	(Rodríguez *et al*., 2014)	β-glucan activates the Unfolded Protein Response in DCs
TGF-α, IFN-r, IFN-α,, IL-29, IL-28B, IL-28A, IL27, IL-23A, IL-19, IL-15, IL-12B, IL-12A, IL10, IL8, IL7, IL-6, IL-1B, IL-1A, EB13, IL22RA1, IL15RA, IL7R, IL6R, IL4R, IL3RA, IL2RA, CCR7, CCR6, CX3CL1, CXCL11, CXCL10, CXCL9, CXCL6, CXCL5, CXCL3, CXCL2, CXCL1, CCL22, CCL20, CCL19, CCL17, CCL15, CCL13, CCL8, CCL5, CCL4, CCL3, CCL1, LILRB4, LAMP3, ECGF1, CD86, CD83, CD80, CD58, CD54, CD47, CD44, ADAMDEC1, ISG20, IFITM3, IFIT4, IFIT2, IFIT1, IFI44, IFI35, IFI27, GIP3, GIP2, RELA, RELB, NFKB2, NFKB1, EB13, CFLAR, BTG1, BASP1, ATF4, AIM2, ADAR, ACPP	Human monocyte-derived DCs from healthy donors	PS-G, a branched (1→6)-β-d-glucan) purified from *Ganoderma lucidum* 10 μg mL^{-1} for 16 h	(Lin *et al*., 2006)	AffymetrixGeneChip microarray
Examples include *RPS18, EGR1, NFKBID, NFATCA1, IFNB1, S100A9, S100A8, GAPDH, TGFA, MMP2, CYP7B1, IL12B, IL12A, EB13, ITGB8, IL27, IL15, CD38, CD80, CCR7, STAT3, IRF1, TNF, CCL4, IL10,IL20, IL23, NFKB1A,NFKBIZ, PTGS2, TLR2, SOD2, NFKB1, IL6, IL1B, IL9*	Human monocyte-derived DCs from healthy donors	β-glucan from baker's yeast (Sigma-Aldrich) 10 μg mL^{-1} 4 or 12 h	(Cardone *et al*., 2014)	Agilent-014850 Whole Human Genome Microarray 4×44 K G4112F GEO accession number GSE42189.
TNF-α, IL6, IL8, IL12, IL18	Human B lymphocytes isolated from healthy donors	Curdlan (Sigma-Aldrich) 100 ug mL^{-1} for 24 h	(Ali *et al*., 2015)	IL-8 expression was shown to involve Dectin-1, SYK, MAPKs and transcription factors AP-1 and NF-kB
GITRL protein	Murine DCs	whole β glucan particles from *S. cerevisiae* treated for 48 h (100 μg mL^{-1})	(Tian *et al*., 2012)	*TNFSF18*is the gene for GITRL, a cytokine in the TNF ligand family
Ddx58, Mda5, IL-1β, IL-6, TNFα, GM-CSF and G-CSF	Murine macrophage-derived RAW264.7 cells	*Aureobasidium pullulans* produced purified β (1->3), (1->6) D glucan 100 μg mL^{-1}) time course experiments: 3-24 h	(Muramatsu *et al*., 2012)	*Ddx58* is RIG-1 (retinoic acid-inducible gene-I). MDA5 is an intracellular pattern recognition molecule
UMPS =Uridine Monophosphate synthetase,	Human KB cells (subclone of HeLa cells) grown as a tumor xenograft in female athymic mice	lentinan purified from *Lentinus edodes* (Shiitake mushroom) Dose (ip injection): 0.1 mg/kg/day 2X/week for 3 weeks	(Harada *et al*., 2010)	measured by RT-PCR in the microdissected tumor; UMPS is also called Orotate Phosphoribosyl Transferase (OPRT)
IFN-Y	Lungs of normal mice	*Antrodia camphorate* beta-glucan; mice were fed beta glucan daily for 12 days	(Wang *et al*., 2015)	

β-D-glucan increased the expression of 9 genes in MCF-7 human breast cancer cells and 5 genes in LCC9 breast cancer cells (Jafaar *et al*., 2014). These genes are summarized in Table 1. Overall, depending on the concentration of β-D-glucan, we observed that β-D-glucan increased the expression of genes involved in proliferation (MK167 (Ki-67), *CCNA1* (cyclin A1) and BIRC5), invasion (*CTSD* (cathepsin D)), inflammation (*PTGS2* (COX-2)), differentiation (PGR (progesterone receptor)), tumor suppression (RASSF1) and DNA repair (*BRCA1, BRCA2*). Surprisingly, a set of different genes were upregulated by β-D-glucan in the endocrine-resistant (tamoxifen and fulvestrant) LCC9 cells with the exception of the common increase in *PTGS2*. These genes were involved in proliferation (EGF, IGF1, IGFBP3) and transcription (GL1). The mechanism for this difference is unknown, although LCC9 cells have higher NFκB expression/activity than MCF-7 (Litchfield *et al*., 2014).

More recent studies reported that two preparations of fungal β-glucans inhibited MCF-7 cell viability, stimulated apoptosis and ROS production and increased necrosis (Queiroz *et al*., 2015). BOT_{GLC} and BOT_{FRU} (100 μg mL^{-1}, Table 1) increased the mRNA and protein expression of p53 (*TP53*) and p27 (*CDKN1B*) after 48 h of treatment (Queiroz *et al*., 2015). The authors reported that BOT_{GLC} activated AMPK, increased FOXO3a protein and reduced phospho-FOXO3a, suggesting that stimulation of FOXO3a transcriptional activity is the mechanism by which cell arrest is achieved. This was supported with the observation that treatment with these β-glucans resulted in an increase in p53 and p27 transcription (Queiroz *et al*., 2015).

β-Glucan Regulates Protein and Gene Expression in Gastric Cancer Cells and other Human Cell Lines

Treatment of human gastric carcinoma cells with β-glucan increased the expression of HSP70 protein (*HSPA1A* gene) and *MUC1* transcript levels (mucin-1, a transmembrane protein) - both of which are protective factors in the gastric mucosa (Tanaka *et al*., 2011). We note that the primary focus of this study was to examine the protective effects of β-glucans on gastric lesions in mice with confirmation of HSP70 and MUC1 in gastric cancer cells.

Synthetic glucans increased expression of *PKC* (protein kinase C), *CXCL8* (IL-8), CDC-42 and PUMA (*BBC3*) transcripts in human Detroit-573 fibroblasts and HaCaT keratinocytes as measured by non-quantitative PCR (Vetvicka *et al*., 2011). These genes are regulators of cell growth and PUMA is a BCL-2 pro-apoptotic protein.

Microarray Analysis in Human Monocyte-Derived Dendritic Cells

Gene array profiling of human monocyte-derived Dendritic Cells (DCs) treated with a purified extract of Polysaccharide (PS-G, a branched (1→6)-β-d-glucan) from *Ganoderma lucidum*, a Chinese medicinal mushroom, identified 3477 (17%) probe sets upregulated (2-fold) and 4418 (19%) probe sets downregulated (2-fold) after 16 h of treatment (Lin *et al*., 2006). The identity of genes included in the text of that manuscript (Lin *et al*., 2006) are shown on Table 1 and 2. Significant increases in transcript levels were observed for a number of cytokines (IL-12A, IL-12B, IL-23A, IL-27 and EBI2; also known as IL-27B), chemokines and chemokine receptors (CCL20, CCL19, CCL5, CXCL10, CXCL11 and CCR7) and cell surface proteins (CD80, CD83 and CD86). The authors also noted that an increase in transcripts for *NFKB1, NBKB2, RELA, RELB and MAPK11*which support the model that β-glucans activate human dendritic cells through the NFκB and p38 MAPK pathways. Further, the authors suggested that the increase in CCL20, IL-27, IL-23A, IL-12A and IL-12B transcripts after PS-G treatment of human DCs may play a role in the anti-tumor activity of β-glucans (Lin *et al*., 2006).

A more recent microarray profiling of human monocyte-derived DCs treated with β-D glucan (10 μg mL^{-1}) for 4 or 12 h identified ~1500 genes that were either inhibited (38%) or induced (62%) (Cardone *et al*., 2014). The β-D glucan-regulated genes were divided into 6 groups: (1) Early-inhibited genes (4-6 h after activation); (2) genes inhibited similarly at both early and late times (early-late-inhibited genes); (3) late-inhibited genes; (4) early-induced genes; (5) genes induced similarly at both early and late times (early-late-induced genes; and (6) late-induced genes (12 h after activation). Gene ontology analysis indicated that the early induced genes were likely involved in apoptosis or stress and antiviral responses, e.g., *IFNB1, NFKBID, NFATC1, TNF, proIL1β, NFKBIZ* and that these genes were potentially regulated by Interferon Regulatory Factors (IRFs). The early-induced genes were identified to be involved in chemotaxis, IL-1 production and NFκB or STAT signaling and were predicted to be regulated by NFκB and IRFs. The late-induced genes included chemokines, cytokines and other factors involved in DC activation and proliferation, e.g., IL6, *I10, IL12B, IL20, IL23A, IL1F9, CSF7, CSF3*; and were predicted to be regulated by NFκB, IRFs, AP1, STATs and CEBPs. The authors demonstrated that β-glucan stimulates IL-1 expression which once processed by the caspase/inflammasome pathway acts in an autocrine fashion to stimulate the expression of the late-induced cytokines at the transcriptional level *via* IκB-ζ modulation (Cardone *et al*., 2014). Further, the authors reported that β-glucan also promoted an IFN-I gene signature consistent with the reported induction of IFN-I in response to fungi *via* Dectin-1 signaling. These data demonstrated that IL-1 and IFN-γ differentially regulate the β-glucan-induced Th responses in human DCs.

Table 2. Genes down-regulated by glucan. The cell type and concentration and form of β-glucan used in these studies is indicated. The comments include information about the genes regulated taken from the references cited and from the Gene Cards human gene database http://www.genecards.org/

Gene	Cell system	β-glucan	Ref.	comments
Measured Cyclin D1 and Cyclin E proteins by flow cytometry	MCF-7 human breast cancer cells	400 μg mL^{-1} PCM3-II, a water-soluble β-glucan purified from the mycelia of *Poriacocos,* 48 h treatment	(Zhang *et al*., 2006)	Cell cycle regulators
CDKN1C, PLAU, RARB	MCF-7 human breast cancer cells	10, 50 μg mL^{-1} β-D glucan; 24 h	(Jafaar *et al*., 2014)	CDKN1C is a negative regulator of cell proliferation; Plasminogen Activator, Urokinase (*PLAU*) is a serine protease, Retinoic Acid Receptor β (*RARB*) is a differentiation maker
CTNNB1, IGFBP2, SLIT2	MCF-7 human breast cancer cells	50 μg mL^{-1} β-D glucan; 24 h	(Jafaar *et al*., 2014)	*CTNNB1*is β catenin; *IGFBP2* is Insulin Like Growth Factor Binding Protein 2*;* SLIT2 is a secreted glycoprotein involved in cell migration
MUC1, SNAI2	MCF-7 human breast cancer cells	10 μg mL^{-1} β-D glucan; 24 h	(Jafaar *et al*., 2014)	MUC1 is a transmembrane protein that is cleaved at its intracellular domain interacts with ERα and contributed to endocrine-resistance (Kufe, 2013); *SNAI2* is the protein LUG which is a transcriptional repressor
ADAM23, BRCA2, CDH13, CDKN1C, CTNNB1	LCC9 endocrine-resistant human breast cancer cells	10, 50 μg mL^{-1} β-D glucan; 24 h	(Jafaar *et al*., 2014)	ADAM23 is a membrane protein that acts as a tumor suppressor; *CDH1* is Cadherin 13; *CDH13 is* Cyclin-Dependent Kinase Inhibitor 1C- a negative regulator of cell proliferation; *CTNNB1* is β-catenin
ERCC5	HepG2 cells	A β-glucan-containing polysaccharide complex purified from *Agaricusblazei* Murill mushroom of Brazilian origin by aqueous extraction (Gonzaga *et al*., 2005) 50 μg mL^{-1} for 6 h	(da Silva *et al*., 2013)	ERCC5 is involved in excision repair following DNA damage
Identified 61 downregulated miRNAs -focused on *mmu-miR-9 CREB mRNA and* protein	Splenic MDSC from tumor-bearing mice	100 ug mL^{-1} for 24 h; whole β-glucan particles from S. *Cerevisiae*	(Tian *et al*., 2015)	miRNA microarray assay GEO accession number GSE67578; Identified Runx1, a transcription factor involved in differentiation of MDSCs, as a direct, *bona fide* target of miR-9. CREB, transcription factor, suppressed miR-9 transcription.
TS, DPD	Human KB cells (subclone of HeLa cells) grown as a tumor xenograft in female athymic mice	lentinan purified from *Lentinus edodes* (Shiitake mushroom) Dose (ip injection): 0.1 mg/kg/day 2X/week for 3 weeks	(Harada *et al*., 2010)	measured by microdissection and RT-PCR; Thymidylate Synthase (TS) Dihydropyrimidine Dehydrogenase (DPD)
ARHGDIB, ATM, CEBPA, CSFIR, CST, F13A1, ITGAM, ITGB2, VCL	Human monocyte-derived DCs from healthy donors	PS-G, a branched (1→6)-β-D-glucan) purified from *Ganoderma lucidum*10 μg/ml for 16 h	(Lin *et al*., 2006)	Affymetrix GeneChip microarray
Ptgs2 (Cox2 gene)	Lungs of normal C57BL/6JNarl mice	*Antrodia camphorate* beta-glucan; mice were fed beta glucan daily for 12 days	(Wang *et al*., 2015)	

β-Glucan Stimulates IL23A and IL-10 Transcription But not IL-12 in Human Dendritic Cells

In a study designed to determine the effect of β-glucan on the production of IL12 and IL23 in human dendritic cells, high and low concentrations of purified β-glucan (10 *versus* 200 μg mL^{-1} zymosan) were shown to induce transcription of IL-23 and IL-10, which was enhanced when cells were simultaneously treated with R848 (a Toll-like receptor

7 and 8 agonist) or with Pam2C (TLR2 ligand) (Gerosa *et al*., 2008). IFN-γ priming inhibited zymosan-stimulated IL-23 transcription (Gerosa *et al*., 2008). In contrast, transcription of IL-12 was not induced by zymosan alone. Instead, expression of IL12 was observed only when cells were first primed with IFN-γ and treated with low concentrations of zymosan and R848. Based on these observations, the authors concluded that the differential expression of IL-23 and IL-12 is dependent on the specific PPRs (pattern recognition receptors) that are activated by different microorganisms. Specifically, TLR2 is able to differentially regulate the expression of these two cytokines which allows DCs to respond appropriately to different pathogens (Gerosa *et al*., 2008).

In a more recent study, Rodríguez *et al*. (2014) showed that selective upregulation of IL-23 by zymosan (1 mg mL^{-1}) involves activating transcription Factor 2 (ATF2) in addition to NFκB which stimulates both IL-23 and IL-10. In these studies, the authors suggest that zymosan interaction with dectin-1 activates PKA-MEK-ERK and PKC signaling leading to activation of ATF2 and coordinates with TLR2 signaling to activate NFκB. ATF2 and NFκB stimulate *IL23A* transcription (Rodríguez *et al*., 2014). While zymogen and two other insoluble β-glucans (curdlan- and pustulan-containing latex beads) resulted in the disappearance of the Endoplasmic Reticulum Stress Response (ERS) transcription factors CHOP and XBP-1 from the nucleus of human DCs and macrophages, there was no evidence that CHOP is involved directly in IL-23 induced expression (Rodríguez *et al*., 2014).

β-Glucans Induce Cytokine Production in Human B Lymphocytes

While most studies have focused on the activation of gene expression in DCs, a recent report demonstrated that purified β-glucan (curdlan) induces expression of several cytokines including TNFα, IL-6 andIL-8 in human B lymphocytes at both the mRNA and protein level (Ali *et al*., 2015). Activation of IL-8 was shown to be mediated through the Dectin-1 receptor and involved SYK, ERK and JNK and activation of the transcription factors AP-1 and NFκB. Interestingly, β-glucan had no effect on antibody production or proliferation suggesting that β-glucans induce a different response than TLR-9 agonist CpG oligodeoxynucleotide (CpG) that is a bacterial and viral DNA component known to induce cytokine production in B lymphocytes (Ali *et al*., 2015).

Glucans Increase gene Transcription in Murine (Mouse) Immune Cells

Treatment of murine Myeloid-Derived Suppressor Cells (MDSCs) with whole β-glucan particles was shown to increase the expression of Runx1, a transcription factor that induces differentiation in Myeloid-Derived Suppressor Cells (MDSC) and *mmu-miR-181d in M-MDSCs (downregulated in G-MDSCs)* (Tian *et al*., 2015). These authors did not examine the function of miR-181d and a search in PubMed revealed no studies examining miR-181d in MDSCs. miR-181d is a stress-responsive miRNA in thymocytes with expression decreasing in response to LPS (Belkaya and van Oers, 2014). A search of microRNA.org revealed 8084 targets for has-miR-181d, but the role of increased miR-181d in response to β-glucan in MDSCs remains to be defined.

Treatment of murine bone marrow derived DCs with Whole β-Glucan Particles (WGPs) rapidly activated the dectin-1 receptor, increased SYK phosphorylation (10-20 min) and increased cell surface GITRL protein (*RNFSF18*) levels measured 48 h later (Tian *et al*., 2012). GIRTL (also called TL6) is a cytokine member of the TNF ligand family. GIRTL primes cytotoxic T lymphocyte responses and downregulates the suppressive activity of regulatory T cells, thus inhibiting tumor progression in the mouse Lewis Lung Carcinoma (LLC) cell model (Tian *et al*., 2012). The authors also showed that treatment of mice implanted with LLC tumor cells with WBPs resulted in an increase in GITRL on DCs *in vivo* and slower tumor development.

Time course experiments in murine macrophage-derived RAW264.7 cells showed that β (1->3),(1->6)-D-glucan, purified from *Aureobasidium pullulan,* strongly upregulated the inflammatory cytokines IL-1β and IL-6 and weakly upregulated TNFα. Increases in GM-CSF (CSF2) and G-CSF (CSF3), cytokines important for differentiation and proliferation, were also observed by 3 h with increased *Ddx58* (RIG-1- retinoic acid inducible gene-1) and *Mda5 (melanoma differentiation-associated protein 5)* seen 6 h after treatment (Muramatsu *et al*., 2012). RIG-1 is necessary for Type I interferon production when cells are infected with a virus and MDA5 is an intracellular pattern recognition molecule for virus-derived RNAs which is necessary for Type I interferon production when cells are virus infected. These results correlate with the anti-viral activity of β-glucan observed in mice (Muramatsu *et al*., 2012).

Treatment of RAW264.7 mouse macrophage cells with β-glucan increased TNFα and GM-CSF (granulocyte macrophage colony stimulating factor) after 3 h. CSF2 is involved in the development and differentiation of lymphocytes from stem cells increased at 3 h and remained upregulated through 24 h.

β-Glucan Regulates Genes in a Human Tumor Cell Xenograft

Treatment of female athymic mice with lentinan (LNT, Glc(b1-3)[Glc(b1-6)]Glc(b1-3)Glc(b1-

3)Glc(b1-3)[Glc(b1-6)]b-Glc, https://pubchem.ncbi.nlm.nih.gov/compound/37723) purified from *Lentinusedodes* (Shiitake mushroom) at a dose of 1 mg/kg/day 2X/week for 3 weeks reduced growth of xenografted human KB cells (a subclone of HeLa cells) (Harada *et al*., 2010). The KB tumors showed reduced expression of UMPS (Uridine monophosphate synthetase, also called Orotate Phosphoribosyl Transferase (OPRT)) at both mRNA and protein levels (Harada *et al*., 2010). Co-treatment with lentinan and S-1, an oral anticancer drug, increased upregulation of UMPS. Inhibition of UMPS would inhibit pyrimidine biosynthesis and thus inhibit nucleic acid biosynthesis, correlating with the anti-tumor activity of lentinan (Ina *et al*., 2013). The authors suggested that since lower levels of expression of OPRT has been associated with resistance to 5-FU based chemotherapies, cotreatment with lentinan may increase the successful use of therapeutic drugs such as S-1 and other 5-FU based chemotherapies.

Genes Inhibited by β-Glucan

A number of studies have identified genes whose expression is decreased by β-glucan treatment in human and mouse cells. Table 2 summarizes the information on these genes that have been identified. First, the results in human cells will be reviewed followed by studies in mouse cells.

β-Glucan Inhibits Gene Transcription in Breast Cancer Cells

Early studies in MCF-7 cells showed that a 48 h treatment with 400 μg mL^{-1} PCM3-II, a water-soluble β-glucan, inhibited cell viability and coordinately reduced Cyclin D1 and Cyclin E protein expression (Zhang *et al*., 2006). However, that study did not examine the impact of PCM3-II on gene transcription. We examined the effect of two concentrations of β-D-glucan on the expression of a set of genes implicated in breast cancer in MCF-7 and LCC9 cells using a PCR array. We reported that β-D-glucan inhibited the expression of cell proliferation and differentiation markers in MCF-7 cells, *e.g*., *CDKN1C, CTNNB1, ERBB2, MUC1* and *RARB* (Jafaar *et al*., 2014). These changes in gene expression correspond to the observation that β-D-glucan dissolved in DMSO, but not water, inhibits MCF-7 cell proliferation (Jafaar *et al*., 2014). β-D-glucan also inhibited the expression of *CDKN1C* and *CTNNB1*in LCC9 cells (Jafaar *et al*., 2014). The mechanism(s) for these effects in MCF-7 and LCC9 cells has not been examined.

β-Glucan Inhibits ERCC5 Expression in HepG2 Cells

Treatment of HepG2 human hepatoma cells with a β-glucan-containing polysaccharide complex purified from *Agaricusblazei* (Murill mushroom of Brazilian origin) by aqueous extraction (Gonzaga *et al*., 2005) had no effect on cell viability (MTT assay), *CYP1A1* or *CASP9* gene transcript levels, but inhibited *ERCC5* expression (da Silva *et al*., 2013). ERCC5 is involved in excision repair following DNA damage, but the authors did not pursue this finding. Interestingly, β-glucan treatment of HepG2 cells increased metabolites involved in bioenergetic metabolism, including alanine, glutamate and creatine. From these studies, the authors concluded that β-glucan increases metabolites needed for high energy metabolism and stimulates bioenergetics (da Silva *et al*., 2013). Interestingly, injection of yellow croaker (*Pseudosciaenacrocea*) fish with β-glucan (5 mg kg^{-1} body weight, from Sigma) 6 h prior to hypoxic stress inhibited reactive oxygen formation in the liver and increased transcription of Pyruvate Kinase (PK) while reducing F-ATPase, Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (MDH) expression (Zeng *et al*., 2016). Which subunit of SDH or form of MDH was examined by PCR was not indicated. These results suggest that β-glucan inhibits the TCA cycle and enhances glycolysis *in vivo* in the liver of yellow croaker fish, although direct examination of TCA cycle metabolites, oxygen consumption or ATP production was not performed.

β-Glucan Inhibits miR-9 Expression in Mouse Macrophage Cells

A microRNA microarray in Whole β-Glucan Particles (WGP)-treated MDSCs isolated from the spleen of Lewis lung carcinoma bearing mice identified 61 miRNAs that were downregulated whereas 40 miRNAs were upregulated (Tian *et al*., 2015). Specifically, the authors identified miR-9 as an important regulator of Runx1 (runt-related transcription factor) as a direct, *bona fide* target of miR-9 (verified by 3'UTR luciferase reporter assay in HEK-293T cells). They also reported a decrease in *Creb* transcript levels in WGP-treated MDSCs, identified a binding site for CREB in the miR-9 promoter region and verified CREB suppression by a promoter luciferase reporter assay. Both CREB and mi-RNA suppression by WGP were shown to be mediated by the Dectin-1 pathway. These results support the conclusion that through the Dectin-1

receptor, CREB directly regulates miR-9 expression in response to β-glucan. The downregulation of miR-9 results in an increase in Runx1 allows for differentiation, decreased immunosuppressive function and reduced tumor growth (Tian *et al*., 2015).

β-Glucan Inhibits Proteins that Regulate Nucleic Acid Synthesis in a Human Tumor Cell Xenograft

As reviewed above, treatment of female athymic mice with lentinan at a dose of 1 mg/kg/day 2X/week for 3 weeks reduced xenograft human KB cell tumors (Harada *et al*., 2010). The authors reported reduced thymidylate synthase and dihydropyrimidine dehydrogenase proteins in the KB xenografts, results that correlated with inhibition of KB xenograft growth *in vivo* (Harada *et al*., 2010).

As discussed above, gene array profiling of human monocyte-derived DCs treated with a PS-G identified 4418 (19%) probe sets downregulated (2-fold) after 16 h of treatment (Lin *et al*., 2006). The identity of downregulated genes included in the text of that manuscript (Lin *et al*., 2006) is shown in Table 2. The authors suggested that the decrease in *ARHGDIB, ATM, CEBPA, CSFIR, CST, F13A1, ITGAM* and *ITGB2* transcripts after PS-G treatment may play a role in the anti-tumor activity of β-glucans (Lin *et al*., 2006). Another microarray profiling of human monocyte-derived DCs treated with β-D glucan (10 μg mL^{-1}) for 4 or 12 h identified ~ 1500 genes that were either inhibited (38%) or induced (62%) (Cardone *et al*., 2014). The authors focused on upregulated transcripts and none of the downregulated genes were described; thus, these are not included in Table 2.

Conclusion

From the genes identified as regulated by β-glucan in this review, it is clear that β-glucan regulates the transcription of numerous immunomodulatory genes in human and mouse dendritic and macrophage cells. In addition, gene expression changes in response to β-glucans have been identified in human breast, gastric and liver cancer cells as well as a few other cell lines as summarized in Table 1 and 2. Figure 1 is a model of the generalized pathway by which β-glucan regulates gene transcription by interaction with a plasma membrane receptor which then activates an intracellular pathways leading to activation or inhibition of transcription factor activity. The activated transcription factors then regulate the transcription of cell-specific target genes.

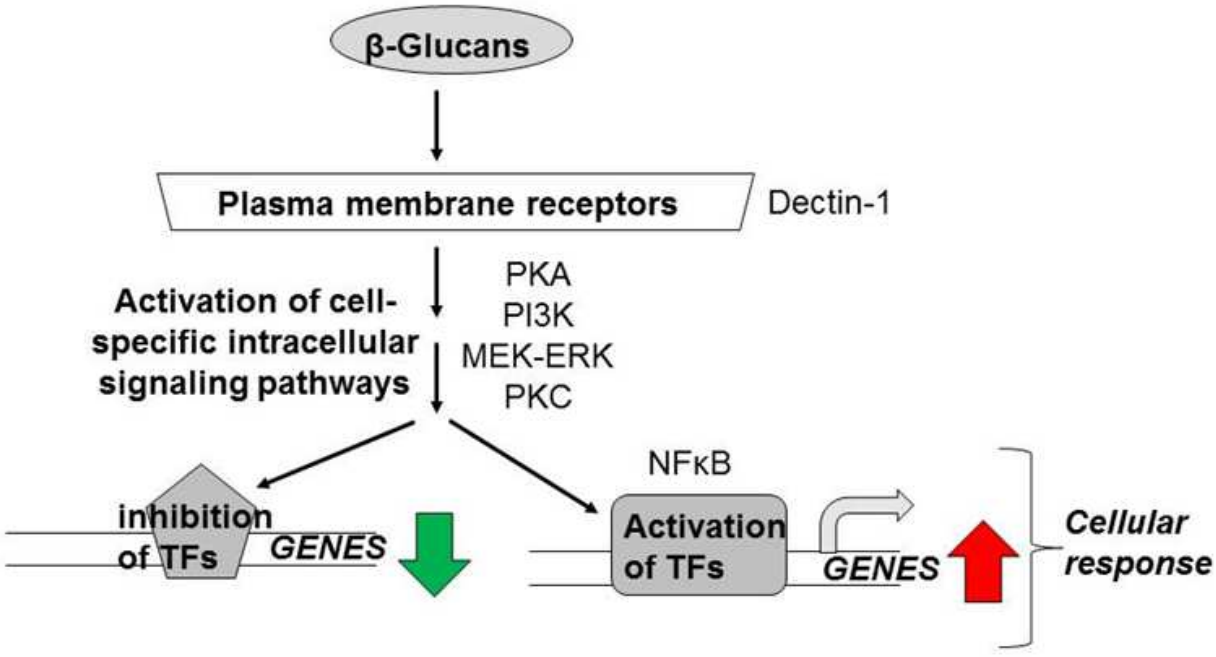

Fig. 1. General model of β-glucan regulation of gene transcription. Examples of cell type specific receptors, pathways and transcription factors are in plain font

Authors Contributions

MOH and CMK contributed equally to the writing of this review.

Conflict of Interest

The authors declare no conflict of interest.

References

Aleem, E., 2013. β-Glucans and their applications in cancer therapy: Focus on human studies. Anticancer Agents Med. Chem., 13: 709-719. DOI: 10.2174/1871520611313050005

Ali, M.F., C.B. Driscoll, P.R. Walters, A.H. Limper and E.M. Carmona, 2015. Beta-glucan-activated human B lymphocytes participate in innate immune responses by releasing proinflammatory cytokines and stimulating neutrophil chemotaxis. J. Immunol., 195: 5318-5326. DOI: 10.4049/jimmunol.1500559

Belkaya, S. and N.S.C. van Oers, 2014. Transgenic expression of MicroRNA-181d augments the stress-sensitivity of $CD4^{+}CD8^{+}$ thymocytes. PLoS One, 9: e85274-e85274. DOI: 10.1371/journal.pone.0085274

Tian, J., J. Ma, K. Ma, B. Ma and X. Tang *et al*., 2012. Up-regulation of GITRL on dendritic cells by WGP improves anti-tumor immunity in murine Lewis lung carcinoma. PLoS One, 7: e46936-e46936. DOI: 10.1371/journal.pone.0046936

Bohn, J.A. and J.N. BeMiller, 1995. (1→3)-β-d-Glucans as biological response modifiers: A review of structure-functional activity relationships. Carbohydr. Pol., 28: 3-14. DOI: 10.1016/0144-8617(95)00076-3

Chan, G., W. Chan and D. Sze, 2009. The effects of β-glucan on human immune and cancer cells. J. Hematol. Oncol., 2: 25-25. DOI: 10.1186/1756-8722-2-25

Cardone, M., A.K. Dzutsev, H. Li, N. Riteau and F. Gerosa, *et al*., 2014. Interleukin-1 and interferon-γ orchestrate β-glucan-activated human dendritic cell programming *via*IκB-ζ modulation. PLoS One, 9: e114516-e114516. DOI: 10.1371/journal.pone.0114516

da Silva, A.F., D. Sartori, F.C. Macedo, L.R. Ribeiro and M.H. Fungaro *et al*., 2013. Effects of β-glucan extracted from Agaricus blazei on the expression of ERCC5, CASP9 and CYP1A1 genes and metabolic profile in HepG2 cells. Hum. Exp. Toxicol., 32: 647-654. DOI: 10.1177/0960327112468173

Gerosa, F., B. Baldani-Guerra, L.A. Lyakh, G. Batoni and S. Esin *et al*., 2008. Differential regulation of interleukin 12 and interleukin 23 production in human dendritic cells. J. Exp. Med., 205: 1447-1461. DOI: 10.1084/jem.20071450

Gonzaga, M.L.C., N.M.P.S. Ricardo, F. Heatley and S.D.A. Soares, 2005. Isolation and characterization of polysaccharides from *Agaricus blazei* Murill. Carbohydr. Pol., 60: 43-49. DOI: 10.1016/j.carbpol.2004.11.022

Harada, K., Y. Itashiki, T. Takenawa and Y. Ueyama, 2010. Effects of lentinan alone and in combination with fluoropyrimidine anticancer agent on growth of human oral squamous cell carcinoma *in vitro* and *in vivo*. Int. J. Oncol., 37: 623-631. DOI: 10.3892/ijo_00000711

Harnack, U., U. Kellermann and G. Pecher, 2011. Yeast-derived beta-(1-3),(1-6)-D-glucan induces up-regulation of CD86 on dectin-1-positive human B-lymphoma cell lines. Anticancer Res., 31: 4195-4199. PMID: 22199280

Ina, K., T. Kataoka and T. Ando, 2013. The use of lentinan for treating gastric cancer. Anticancer Agents Med. Chem., 13: 681-688. DOI: 10.2174/1871520611313050002

Jafaar, Z.M., L.M. Litchfield, M.M. Ivanova, B.N. Radde and N. Al-Rayyan *et al*., 2014. Beta-D-glucan inhibits endocrine-resistant breast cancer cell proliferation and alters gene expression. Int. J. Oncol., 44: 1365-1375. DOI: 10.3892/ijo.2014.2294

Kim, H.S., J.T. Hong, Y. Kim and S.B. Han, 2011. Stimulatory effect of β-glucans on immune cells. Immune Netw., 11: 191-195. DOI: 10.4110/in.2011.11.4.191

Kufe, D.W., 2013. MUC1-C oncoprotein as a target in breast cancer: Activation of signaling pathways and therapeutic approaches. Oncogene, 32: 1073-1081. DOI: 10.1038/onc.2012.158

Legentil, L., F. Paris, C. Ballet, S. Trouvelot and X. Daire *et al*., 2015. Molecular interactions of β-(1-->3)-glucans with their receptors. Molecules, 20: 9745-9766. DOI: 10.3390/molecules20069745

Li, B., Y. Cai, C. Qi, R. Hansen and C. Ding *et al*., 2010. Orally administered particulate β-glucan modulates tumor-capturing dendritic cells and improves antitumor t-cell responses in cancer. Clin. Canc Res., 16: 5153-5164. DOI: 10.1158/1078-0432.CCR-10-0820

Lin, Y.L., S.S. Lee, S.M. Hou and B.L. Chiang, 2006. Polysaccharide purified from *Ganoderma lucidum* induces gene expression changes in human dendritic cells and promotes T helper 1 immune response in BALB/c mice. Mol. Pharmacol., 70: 637-644. DOI: 10.1124/mol.106.022327

Litchfield, L.M., S.N. Appana and C.M. Klinge, 2014. COUP-TFII inhibits NFkappaB activation in endocrine-resistant breast cancer cells. Mol. Cell Endocrinol., 382: 358-367. DOI: 10.1016/j.mce.2013.10.010

Lyakh, L., G. Trinchieri, L. Provezza, G. Carra and F. Gerosa, 2008. Regulation of interleukin-12/interleukin-23 production and the T-helper 17 response in humans. Immunol. Rev., 226: 112-131. DOI: 10.1111/j.1600-065X.2008.00700.x

Muramatsu, D., A. Iwai, S. Aoki, H. Uchiyama and K. Kawata *et al*., 2012. β-Glucan derived from *Aureobasidium pullulans* is effective for the prevention of influenza in mice. PLoS One, 7: e41399-e41399. DOI: 10.1371/journal.pone.0041399

Novak, M. and V. Vetvicka, 2008. β-glucans, history and the present: Immunomodulatory aspects and mechanisms of action. J. Immunotoxicol., 5: 47-57. DOI: 10.1080/15476910802019045

Queiroz, E.A.I.F., Z.B. Fortes, M.A.A. da Cunha, A.M. Barbosa and N. Khaper *et al*., 2015. Antiproliferative and pro-apoptotic effects of three fungal exocellular β-glucans in MCF-7 breast cancer cells is mediated by oxidative stress, AMP-Activated Protein Kinase (AMPK) and the Forkhead transcription factor, FOXO3a. Int. J. Biochem. Cell Biol., 67: 14-24. DOI: 10.1016/j.biocel.2015.08.003

Rodríguez, M., E. Domingo, S. Alonso, J.G. Frade and J. Eiros *et al*., 2014. The unfolded protein response and the phosphorylations of activating transcription factor 2 in the trans-activation of il23a promoter produced by β-glucans. J. Biol. Chem., 289: 22942-22957. DOI: 10.1074/jbc.M113.522656

Ross, G.D., 2000. Regulation of the adhesion versus cytotoxic functions of the Mac-1/CR3/alphaMbeta2-integrin glycoprotein. Crit. Rev. Immunol., 20: 197-222. PMID: 10968371

Saraswat-Ohri, S., A. Vashishta, V. Vetvicka, K. Descroix and F. Jamois *et al*., 2011. Biological properties of (1 → 3)-β-d-Glucan-based synthetic oligosaccharides. J. Med. Food, 14: 369-376. DOI: 10.1089/jmf.2010.0081

Tanaka, K., Y. Tanaka, T. Suzuki and T. Mizushima, 2011. Protective effect of β-(1,3 → 1,6)-d-glucan against irritant-induced gastric lesions. Br. J. Nutr., 106: 475-485. DOI: 10.1017/S0007114511000365

Tian, J., K. Rui, X. Tang, J. Ma and Y. Wang *et al*., 2015. MicroRNA-9 regulates the differentiation and function of myeloid-derived suppressor cells via targeting Runx1. J. Immunol., 195: 1301-1311. DOI: 10.4049/jimmunol.1500209

Vetvicka, V., S. Saraswat-Ohri, A. Vashishta, K. Descroix and F. Jamois *et al*., 2011. New 4-deoxy-(1→3)-β-d-glucan-based oligosaccharides and their immunostimulating potential. Carbohydr. Res., 346: 2213-2221. DOI: 10.1016/j.carres.2011.06.020

Wang, W.J., Y.S. Wu, S. Chen, C.F. Liu and S.N. Chen, 2015. Mushroom beta-glucan may immunomodulate the tumor-associated macrophages in the Lewis lung carcinoma. Biomed. Res. Int., 2015: 604385-604385. DOI: 10.1155/2015/604385

Zeng, L., Y.H. Wang, C.X. Ai, J.L. Zheng, C.W. Wu *et al*., 2016. Effects of β-glucan on ROS production and energy metabolism in yellow croaker (*Pseudosciaena crocea*) under acute hypoxic stress, Fish Physiol. Biochem., 42: 1395-1405. DOI: 10.1007/s10695-016-0227-1

Zhang, M., L.C. Chiu, P.C. Cheung and V.E. Ooi, 2006. Growth-inhibitory effects of a beta-glucan from the mycelium of Poria cocos on human breast carcinoma MCF-7 cells: Cell-cycle arrest and apoptosis induction. Oncol. Rep., 15: 637-643. PMID: 16465424