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Minireview

Versatility of berberine as an effective immunomodulator and chemo sensitizer against p53 mutant cell

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Cancer is the leading cause of death among individuals due to its poor prognosis. Various therapeutics treatments are available in form radiation therapy, chemotherapy, or immunotherapy but major point of concern is the treatment of cancer resistant cell lines. Homozygous loss of the p53 gene is virtually present in every type of cancer. Mutation in DNA binding domain of p53 leads to formation of mutant forms having altered amino acid sequence which lacks DNA binding activity. Berberine is chemo-sensitizing isoquinoline quaternary alkaloid molecule obtained from *Berberis vulgaris*. Berberine has the capability to suppress the growth of broad range of tumors. It exhibits pharmacological, biochemical and anticancer properties which can potentiate the activities of the existing therapeutics available in a way that it can re-sensitize the cancer resistant clones. Berberine has an immanent potential to bind with DNA and can communicate with several cellular targets, further it also shows hormetic effect which refers to biphasic dose response curve in order to determine dose dependent stimulatory and inhibitory effect. Mode of action involved is yet not well understood but mechanistic pathway involved are autophagy, up-regulation of tumor-suppressor gene (p53) and epigenetic alterations in the viral DNA. In this review, versatility of berberine can be utilized ideally or in combination with chemotherapeutics drugs to potentiate chemo sensitization of the resistant cancer cell line. Further, cancer cell specific receptor targeting can also be employed in combination with berberine for therapeutic treatment of metastasizing cancer cells.

Keywords: Berberine, Chemo-sensitizing, Epigenetic alterations, Hormetic effect, Tumor-suppressor gene

Introduction

Cancer is one of the common deadliest disease occuring amongst individuals because of its poor prognosis and advances in its recurrence. According to global statistic report on regional and national cancer incidence, it has been reported that breast cancer, lung cancer, prostate cancer, liver cancer, ovarian cancer *etc*. are listed amongst the top economic burdens[1]. In 2020, population-based cancer registries (PBCR) provided the projected incidence of cancer that reveals 679,421 male individuals and 712,758 female individuals were affected in India[2]. Increasing risk of cancer incidence and cancer related early death of an individual also depends on human development index (HDI) of that country. India, a country of medium HDI index is reported with increasing cancer incidence cases. Sex specific cancer case studies in male individuals reveal increasing rate of lip and oral cancer in India and prostate cancer globally while for female individuals breast cancer cases are reportedly higher in India as well as worldwide[3]. In cancer, unregulated abnormal growth of cells is characterized by loss of contact inhibition, evaded programmed cell death mechanism, infinite cell multiplicity and sustained angiogenesis, invasion and metastasis capabilities[4]. Cancer occurrence and its subsequent succession are due to accrual of mutations with time, either due to loss or gain of function. Presence of functional loss of wild type tumor

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Abbreviations: ABC, ATP-Binding Cassette; AIF, Apoptosis Inducing Factor; BRB, Berberine; CDK, Cyclin Dependent Kinase; COX-2, Cyclo-oxygenase-2; CXCLs, Chemokines; DNMT, DNA Methyl Transferase; ECM, Extra Cellular Matrix; EMT, Endothelial Mesenchymal Transition; HDAC, Histone Deacetylase; HDI, Human Development Index; HNF4a, Hepatocyte Nuclear Factor; HO-1, Heme-oxygenase-1; iNOS, inducible Nitric Oxide Synthase; MDR, Multidrug Resistance; MiRNA, micro-RNA; MMP, Metalloprotease; MMR, Mismatched Repair; Mutant p53, Mutant tumor suppressor gene; NQO-1, Nicotinamide Adenine Dinucleotide Phosphate Quinone Oxidoreductase; PBCR, Population Based cancer resistance; p-gp, P-Glycoprotein; PI3K, Phosphoinositide 3-Kinase; PKC, Protein Kinase C; ROS, Reactive Oxygen Species; RXRα, Retinoid X-Receptor α; TAA, Tumor Associated Antigens; TME, Tumor Micro-Environment; TNBC, Triple Negative Breast Cancer; T-reg cells, T-regulatory cells; TSA, Tumor Specific Antigens; VEGFR, Vascular Epidermal Growth Factor Receptor; WTp53, Wild type tumor suppressor gene

suppressor gene (WTp53) organ of functional mutation as mutant tumor suppressor gene (Mp53)[5], both aim to augment cancerous growth. In majority of cancer cases chief pathway allied with tumor suppression are identified with dysfunctional regulation of cancer malignancy represented as initiated cell death mechanism, cell cycle arrest at check points, apoptosis initiation in cancer cells and activation of DNA repair mechanism[6]. Thus, functional abolition of p53 gene can be considered to be an alleged key feature of majority of cancers. Further it can be stated that functional loss in wild type tumor suppressor gene (WTp53) expression is due to mutation which leads to formation of mutant p53 (Mp53). This shift in p53 expression exhibits oncogenic properties *via* dominant negative repression of wild type p53activity[7].

Apart from surgery various other standardized therapeutics for cancer treatments are represented as follows: chemotherapy, radiotherapy, immunotherapy, hormonal therapy, combination therapy *etc*. But due to futile attempts these therapies cannot come up to the expected survival rate in cancer patients. In the recent past the chief emerging reasons for morbid survival rate in cancer patients can be in form of acquired survival strategies by cancer cells against therapeutic regimes referred to as cancer acquired resistance and oncogenic shift in the host immune cells expression within tumor microenvironment (TME) responsible for immune suppression. Both of them aim to fuel up cancer sustenance and progression[8]. Shaping of the sensitive cancer cells to resistant type is a resultant of obscured sensitivity of cancer cells due to cells native capability responsible for reducing effectiveness of there gimes in form of chemotherapeutic drug, ionizing radiation *etc*. This resistance can also be acquired during the course of treatment which enables cancer cells to escape from cytotoxic effect of the therapeutics[9]. Other than this ability of cancer cells to escape from host immune surveillance through inadequate immune mediated infiltration, immune response shift toward protumor immunity, immune suppression, enhanced chronic inflammation, extracellular matrix (ECM) remodeling *etc*. due to differential crosstalk among tumor promoting cells and tumor inhibiting cell within TME[10]. These issues need to be resolved in order to enhance the effectiveness of therapeutic regime and to decreases morbidity rate in cancer patients.

Thus, there is pre-requisite requirement of such natural compounds which has ability to potentiate chemotherapeutic properties of available anticancer drugs and also has role as an immune modulator. Natural compound with such properties can be utilized for treatment against cancer malignancies. Phytochemicals is one such group, refers to class of secondary metabolites derived from plants that includes a wide range of alkaloids, flavonoids, glycosides, gums, polysaccharides, phenols, tannins, terpenes, and terpenoids *etc*. employed for this purpose. Berberine (BRB) is one such phytochemical extract which belong to genus *Berberis*. This genus further involves diverse range of species with respective amount of BRB present in it- *Berberis aristata* 5% in roots and 4.2% in stem-bark, *Berberis petiolaris* 0.43%, *Berberis vulgaris* 1.24%, *Berberis aquifolium*, *Berberis thunbergii*, *Berberis asiatic*, *Coptisteeta* 8-9% in rhizome *B. lycium*is one such species present throughout Himalayan range in India[10-11]. *Berberine vulgaris* is isoquinoline quaternary alkaloid molecule obtained from *Berberis vulgaris.* In this review of literature various properties of BRB is depicted in a form, so that it can potentially be utilized as chemosensitizer and immunomodulator, thus down-regulating cancer promoting expression at gene or protein level by targeting mutant p53 in a way providing enhanced survival rate in cancer patients.

Tumor suppressor gene and tumorigenic shift in its expression

Tumor suppressor gene (p53) and its suppressive action against tumor growth is long been reported. p53 is specifically known to regulate cell-cycle arrest at specific check points, induces cell death pathways and activates DNA repair mechanism. Chief work allied to tumor suppressor gene has focused on functional aspects of p53, p53 degradation pathway and its downstream related signaling pathway inhibiting cancerous growth[12]. Its chemo-sensitization and immune regulatory role is predetermined regulated by various signaling pathway. WTp53 mediated chemo-sensitization includes inhibition of the drug efflux transporter (ATP-binding cassette (ABC)) whose prime liability is concerned with initiation of multidrug resistance with binding to multidrug resistance (MDR) promoter region[13], it also targets transition from epithelial-mesenchymal state with in cancer cells[14] along with DNA repair related mechanism (mismatch repair mechanism (MMR) *etc*[15] as its constitutive expression or acquired mutation constrain the sensitization pattern in cancer cells. Regulatory role of p53 mediated pathway downstream also regulates production of pro-inflammatory molecules such as cytokines, cyclooxygenase 2 (Cox-2), and

inducible nitric oxide synthase (iNOS) *etc*[16]. Immune regulatory role of p53integrate host innate and adaptive immune response this includes recognition of tumor associated antigens (TAAs) or tumorspecific antigens (TSAs) expressed on the surface of cancer cells failure attempt of which is associated with cancer metastasis and progression[17]. Role of innate and adaptive immune cells (macrophages, dendritic cells, natural killer cells, cytotoxic tlymphocyte, B cell *etc*.) in cancer cells elimination is well documented but immune suppression or acquired immune tolerance enables cancer cells to escape from host immune surveillance. Other than this oncogenic shift in immune cells expression also stimulates tumor progression over the period of time[18]. Loss of p53 expression as per mutational event is key marker for failure of functional activity as acquired resistance and protumor immunity against invasive and progressive cancer cells[19].

Majority of cancers are marked by altered expression of tumor suppressor gene p53. Tumorigenic change in expression of p53 is due to missense mutations in p53 gene represented as contact mutation (R248 or R273 residues) or conformational mutation (R175 or H179 residues). Mutation in amino acid is involved at DNA protein interaction, it is responsible for mutant p53 stability while mutation at DNA-binding domain exhibit functional loss of WTp53expression or its trans dominant repression *via* hetero-tetramer formation with WTp53[20].

Versatility of berberine

Chemical structure, bioavailability and biodistribution of berberine

Berberine ($C_{20}H_{18}NO_4^+$) chemical name-1, 3-Benzodioxolo[5,6-a]benzo[g]quinolizinium, 5,6-dihydro-9,10-dimethoxy; is versatile heterocyclic, nitrogen containing isoquinoline alkaloid molecule (Fig. 1). It is isolated from the roots, rhizomes, stemand barks of many medicinal plants, one such plant is *Berberis vulgaris* (barberry) which belongs to family Berberidaceae. Its use in medicinal form has been reported in Chinese and Indian Ayurvedics system for centuries[10].

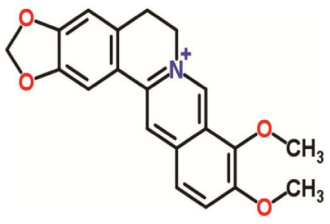

Fig. 1 — Chemical structure of berberine

Bioavailability of berberine is very poordue to its hydrophobic nature, that retard its intestinal absorption and leads to fast elimination from body. Through P-glycoprotein (P-gp) efflux pump which are responsible for its removal from physiological system. Further biodistribution of BRB reveals specific localization order in various organs represented as follows: kidneys, muscles, lungs, brain, heart, pancreas, and fat[21].

Versatile nature of berberine

Berberine plays a critical role as an anticancer, anti-inflammatory agents, cell cycle regulator, by facilitating the reactive oxygen species (ROS) production or by regulating the cellular signaling pathway, it also acts as an intercalating agent, that participates in DNA damage[22]. Further it has been reported that structural modifications at different carbon position makes it's more versatile such as substitution at C-8, C-9, and C-13 position increases its antitumor activity. Structure-activity relationship patterns reveal more effectiveness of alkyl group than benzyl group in substituted BRB derivatives as length of carbon chain determines its effective rolein treatment efficacy for cancer therapeutics. It also states that addition of ester, amides or sulfonates also enhances it by potentiating it response curve [23]. These therapeutics properties of BRB have gathered much attention that focus towards its immune-modulatory and chemo-sensitizing abilities.

Properties of berberine, molecular targets and mechanism of action

BRB an anticancer agent (Fig. 2) target cancer cells as an apoptotic/autophagy inducer in caspase dependent and independent manner thus, initiating cell death mechanism within cancer cells. In caspase dependent pathway it tends to restore normal Bax/Bcl-2 ratio by direct action on pro apoptotic factor (Bax, Bad, caspase3, caspase9 *etc*.) thus significantly increases its level followed by simultaneous decreases in various anti apoptotic (Bcl-2, c-IAP1, Bcl-xL) factors, further in caspase independent apoptotic pathway (ROS dependent pathway) cellular ROS production induces activation of AIF (apoptosis-inducing factor) resulting in apoptotic death. Here, BRB up-regulates the production of cellular ROS *via* JNK/p38 MAPK or calcium dependent protein kinase C (PKC)[24]. Role of BRB in p53 dependent (WT p53) and independent pathway (Mp53) of apoptosis is well described in

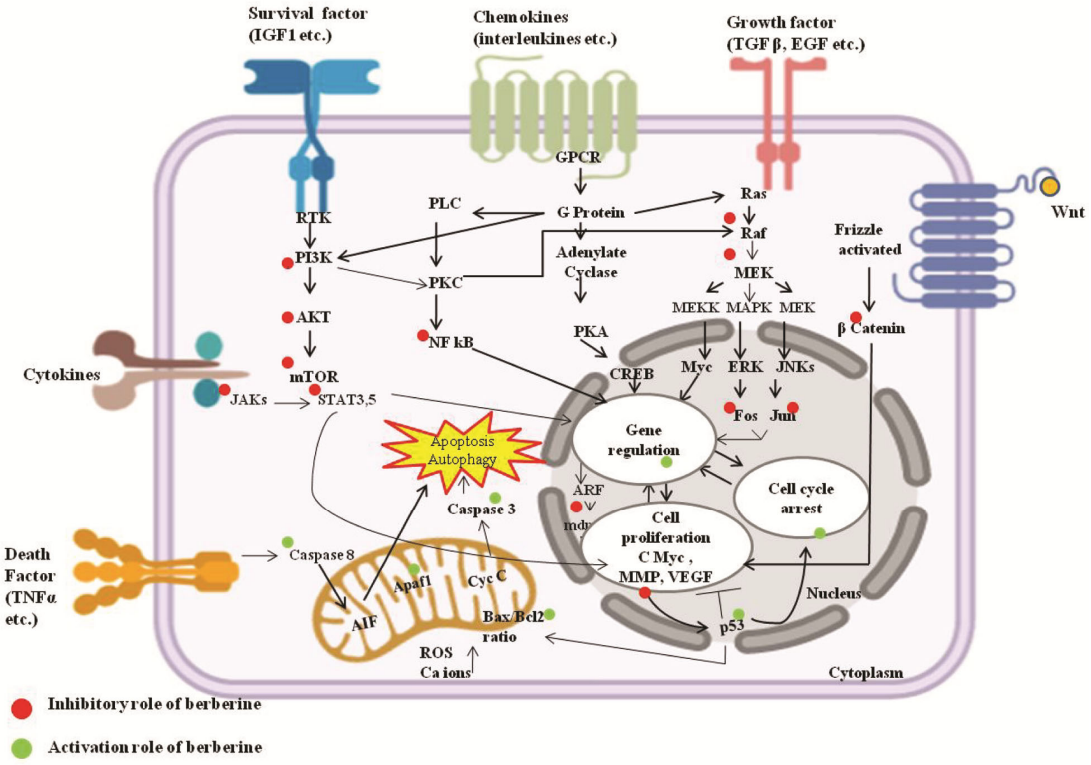

Fig. 2 — Schematic representation of molecular targets of berberine and mode of action

breast cancer cell line MCF-7 and MDA-MB-231[25]. BRB intervene cancer cells progression by inducing cell cycle arrest, it targets cyclin D1, cyclin E and cyclin dependent kinase (CDK)- Cdk2, Cdk4 and Cdk6 expression levels at G0/G1 phase of the cell cycle. This is also referred as p53 dependent regulation because p53 mediated up-regulation of p21mRNA and protein levels is responsible for cell cycle arrest by targeting CDK activity. p53 independent arrest at G2/M phase of cell cycle is also reported in various cancer studies[25]. Targeting signaling pathways such as COX-2/PGE2axis directly inhibit cytoplasmic cyclooxygenase-2 (COX-2) downstream attenuates JAK2/STAT3 signaling pathway responsible for cancer cell invasion & metastasis by increasing matrix metalloprotease expression MMP2, MMP9, vascular endothelial growth factor receptor (VEGFR) expression *etc*. PI3K-AKT/ERK pathway is also known to be involved in oncogenic progression and sustenance, BRB here suppresses phosphorylated expression levels of PI3K/AKT in various cancers[26], thus, minimizes risk related to cancer progression. BRB also targets WNT/β-catenin signaling pathway at constitutive nuclear β-catenin expression which are known for deviating it's functioning towards cancer specific gene expression represented with elevated level of genes specific to tumor vascularization/metastasis, inflammatory cytokines *etc*[24,26]. BRB binding to ligand binding domain of retinoid X receptor alpha (RXRα) nuclear receptor at Gln275, Arg316 and Arg371 residue enables its interaction with nuclear β-catenin thus suppressing its expression *via* c-Cbl mediated proteasomal degradation of β-catenin[27]. BRB also obstructs tumor metabolic pathway which tends to meet the energy demand within tumor cells. Studies indicate role of nuclear receptor hepatocyte nuclear factor 4a (HNF4a) in glucose and lipid metabolism, which downstream increases expression level of HIF-1a. BRB suppresses metabolic pathway supply by acting as AMPK activator, phosphorylated active AMPK act *via* AMPK/HNF4a pathway[27]. Various other cancer related signaling pathway such as PI3K/Akt (phosphoinositide 3-kinase/Akt), Ras/Erk pathway and MAP (Raf/MEK/ERK) pathway *etc*. are all reported to be targeted by berberine. In recent it has also been reported that presence of berberine reverses methylation induced silencing of gene expression by targeting DNA methyl transferase(DNMT1,

DNMT3A, DNMT3B) in cell line specific manner[27]. It's interaction with histone deacetylase 1 (HDAC1) & histone deacetylase 2 (HDAC2) is reported due to presence of BRB binding site on it. Molecular docking studies states that Arg and Glu rich regions are more appropriate site to interact with HDAC1 and HDAC2[28]. This study presents the new insight to improve cancer therapeutics by utilizing BRB to regulate expression of various silenced anti-tumorigenic gene by targeting epigenetic specific marker thus playing key role as an epigenetic regulator. Anti-inflammatory and anti-oxidative role of BRB relies on Nrf2 activation mediated through AMPK, PI3K/Akt and P38 pathway activation. BRB being AMPK activator obstructs MAPK pathway in AMPK dependent manner, inhibits NF-κB activation, attenuates AP1 expression upstream that is regulated by PPARγ activation thus, targets inflamed immune response that are regulated through activation of various inflammatory genes (inducible nitric oxide synthase (iNOS), COX-2). It also up-regulates the expression of Nrf2 specific anti-oxidative genes such as nicotinamide adenine dinucleotide phosphate quinone oxidoreductase-1 (NQO-1), heme oxygenase-1 (HO-1) *etc*. Immune suppressive action of berberine can effectively targets the release of various cytokine such as IL-2, TNF-α, and IFN-γ *etc*[29]. Therefore, it can also be presumed to have immune activation role, overcoming the conventional issues related to suppressed immune response.

Berberine related chemo-sensitization and immune modulation mechanism in cancer cells

Berberine as an effective chemosensitizer molecule

Berberine target drug transporters

Interaction of berberine with drug transporter reveals BRB binding site on P-glycoprotein (P-gp) at subordinate end of drug binding pocket thus, interaction of BRB tends to act as a key regulator for drug transportation at both mRNA and protein levels in various cancer cells such as HepG2 cells, HeLa and SY5Y cells. BRB show differential effect on P-gp expression as it inhibits P-gp in HepG2 cells, and *vice versa* reported in HeLa and SY5Y cells. Role of BRB as an effective chemosensitizer for cancer is manifested through various multidrug resistant cancer studies this includes oral cancer (KB, OC2), gastric cancer (SC-M1, NUGC-3) and colon cancer (COLO 205, CT 26)) where it stimulates P-gp expression[30]. It has been reported that subsequent treatment of BRB along with anti-metabolite 5-Fluorouracil also decreases the MDR expression in lung cancer (A549) thus minimizes the activity at drug efflux pump and enhances the retention of drug within cancer cells[30].

Berberine targets DNA damage repair mechanism

Resistant cancer mediated acquired survival strategy impede the therapeutic efficacy of anticancer drugs. BRB treated cisplatin resistant ovarian cancer(SKOV3) has been reported with restoration of demethylation status in human mutL homolog gene (hMLH1) with up-regulation of hMLH1 mRNA level which encodes for DNA mismatch repair mechanism specific protein in time and dose dependent manner[31].

Berberine mediated activation of apoptotic pathway

In multi-drug resistant cancer cells such as lung cancer (A549) BRB along with 5-Fluorouracil (5-FU)/Camptothecin (CPT) and its analog irinotecan (CPT-11) it increases ROS level[32]. Antitumor effect of BRBalong with tamoxifen in both sensitive and resistant breast cancer (MCF-7) it increases cytotoxic activity due to p21Cip-1 initiation marked with elevated BAX/BCL2 ratio[33].

Berberine reverses epithelial to mesenchymal transition (EMT)

BRB act against resistance acquired epithelial to mesenchymal transition by targeting EMT specific marker in dose dependent manner. It up-regulates the expression of epithelial marker such as E-cadherin and limits the expression of mesenchymal marker N-cadherin which is specific for EMT as reported in cervical cancer, this can be achieved by targeting EMT specific factors such as SNAIL1[33,34]. BRB reverse EMT at TGF β/Smads signaling pathway in normal colonic epithelial cells (HCoEpiCs), in colon cancer (SW480), resistant lung cancer (A549) *etc*. further BRB in cisplatin resistant ovarian cancer EMT is regulated *via* miR-21/PDCD4 axis[35].

Berberine mediated regulation of targeting expression of various genes & transcription factors

BRB regulates expression of various transcriptional activators such as NF-κB, AP-1, STAT3 *etc*. that are involved in cancer related pathway as they are tumor growth promoting signal that are needed to be suppressed. There constitutive expression leads to up-regulation of cancer angiogenesis, proliferation, metastasis *etc*. BRB target NF-κB, decreases its constitutive expression thus chemosensitizes resistant breast cancer, lung cancer, colon cancer, prostate cancer *etc*. BRB targets c FOS and JUN D expression in a way that it inhibits AP-1 complex formation thus,

targeting AP1 activity a possible mechanism of chemo-sensitization in cervical cancer where it acts to suppress AP-1 activity. Similarly, it can also be applying to other cancers too[36]. In lung cancer BRB induces sensitivity against doxorubicin by suppressing STAT3 transcriptional and translational activity *via* protein ubiquitination or *via* suppression of STAT3 phosphorylation at Tyr705. Other than this it also suppresses downstream signaling element such as c-Myc[37]. BRB can also act as a radiosensitizer as reported from various *in vitro* and *in vivo* studies where it enhances the radiosensitivity *via* inhibition of VEGF and HIF-1α in esophageal cancer[38] (Fig. 3).

Berberine as an effective immunomodulatory and adjuvant molecule

Berberine mediated immunomodulatory shift in macrophage polarity

Anti-tumorigenic to pro-tumorigenic switch in macrophage activity is due to macrophage polarization from classical macrophage (M1ϕ) to alternative macrophage (M2ϕ) phenotype. Tumor associated macrophage (TAM) belongs to M2ϕ specific subtype. Macrophage and BRB related studies reveal reduced tumor invasion/migration in intestinal tumorigenic mice model (treated with dextran sulfate sodium) after subsequent treatment with BRB here, it suppresses colitis associated tumor development *via* inhibition of IL-6 & TNF-α release from colonic macrophage[39]. Initially it was thought to be NOX2 expression in M2ϕ macrophage which is targeted by BRB but later studies reveal that BRB suppresses macrophages polarization *via* COX-2 pathway during inflammation or by reversing M1ϕ toM2ϕ macrophage polarization *via* targeted action on NADPH Oxidase/ROS/COX-2 signaling pathway. Thus, BRB here tends to act as a modulator molecule screwing macrophage phenotype for effective tumor targeting[40]. Other than this EMT specific transcriptional factor Snail also enhances M2ϕ macrophage phenotype *via* activation of MIR21 expression which increases secretion of miR-21, its abundance enhances the presence of tumor-

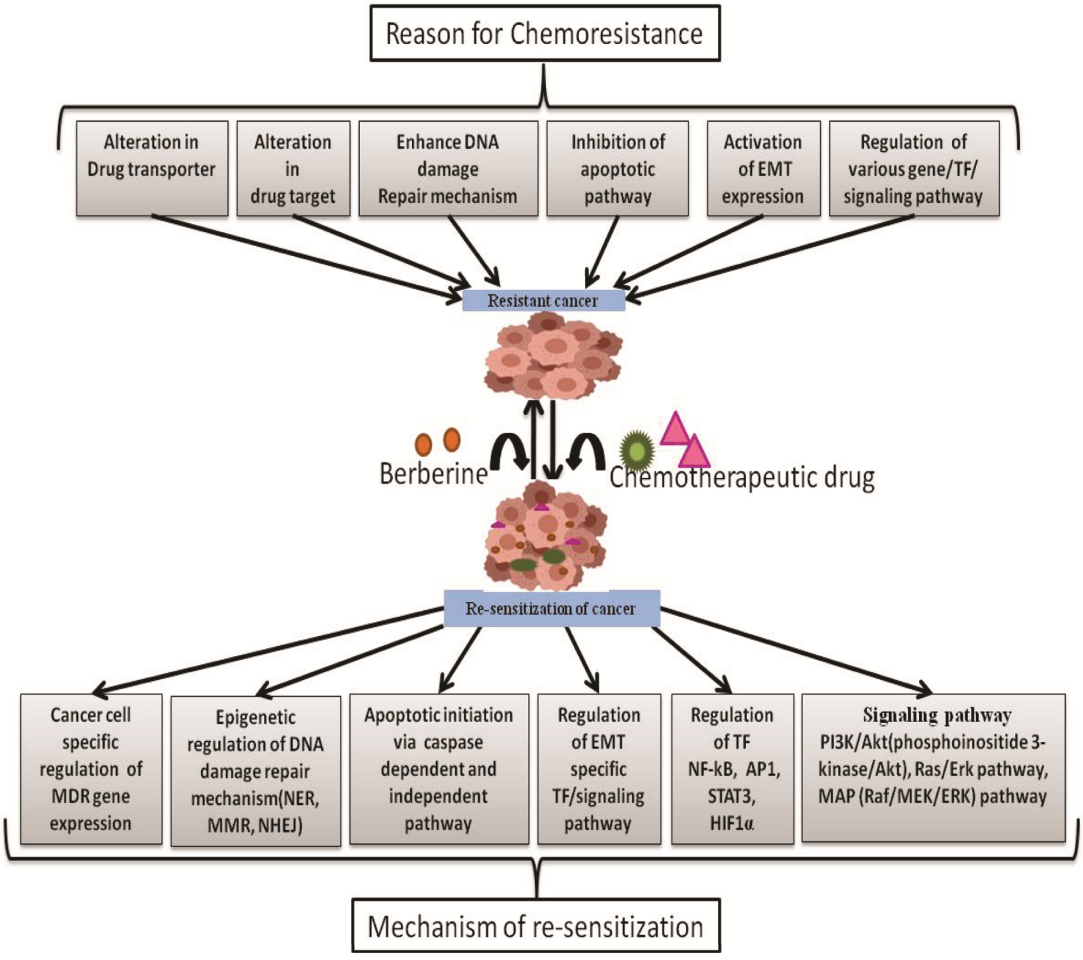

Fig. 3 — Schematic representation of reason for chemoresistance and berberine as effective chemosensitizer

derived exosomes in monocytes, further it aims to suppress M1ϕ specific markers expression and promote surface expression of M2ϕ specific markers. BRB mediated inhibition of epithelial to mesenchymal transition can hold the M1ϕ macrophage in its native functional conformation contributing to antitumor immunity[41] (Fig. 4).

Berberine as immunomodulator for lymphocyte polarization

BRB is reported to suppress inflammatory response in type 1 diabetes by inhibiting differentiation of Th17 and Th1 *via* activation of ERK1/2 and inhibition of p38 MAPK and JNK activation. Further down-regulated STAT3 & STAT1/STAT4 expression downstream effectively inhibits Th1 polarization[42]. In inflammatory bowel disease BRB inhibit the differentiation of Th17/Th1 *via* MAPK pathway while it does not have any significant effect on Tregulatory cells (T reg cells) expression. BRB shifts the Th1/Th2 balance toward the Th2 polarization reported in LPS induced mouse primary splenocytes[43]. This polarization shift can be skewed towards Th1 polarization, directing it towards Tcytotoxic cells and anti-tumorigenic M1ϕ macrophage activation. This skewness can also be achieved by targeting Type 2 Neutrophils (N2) known for its immunosuppressive phenotype through Th2-type mediated inflammation within TME. BRB is known to induce ROS mediated autophagy in Type 2 Neutrophils (N2) thus retain the existence of antitumor Type 1 Neutrophils (N1) within TME[44].

Berberine mediated targeting of other immune cells

Targeting Treg cells expression can shift immune response curve in cancer patients. BRB mediated direct regulation of Treg cells expression is not known but indirectly it's expression can be regulated by various chemo-attractants such as chemokines (CXCLs), cytokines (TNF-α, ILs, TGF-β, *etc*.) specially TGF-β which are responsible for immune suppression, macrophage polarity shift *etc*[45]. Various *in vitro* studies reveals that BRB can regulate inflamed immune response by targeting release of cytokine/chemokine or by regulating signaling expression at tumor cells , pro-tumorigenic immune cells (Neutrophils (N2), TAM, Treg cells *etc*.) within TME. BRB directly targets constitutive expression of STAT-3 in nasopharyngeal carcinoma. It also inhibits cytokine (IL-6) mediated active STAT3 expression in CAF in nasopharyngeal carcinoma[46]. IL-8 secretion is known to promotes

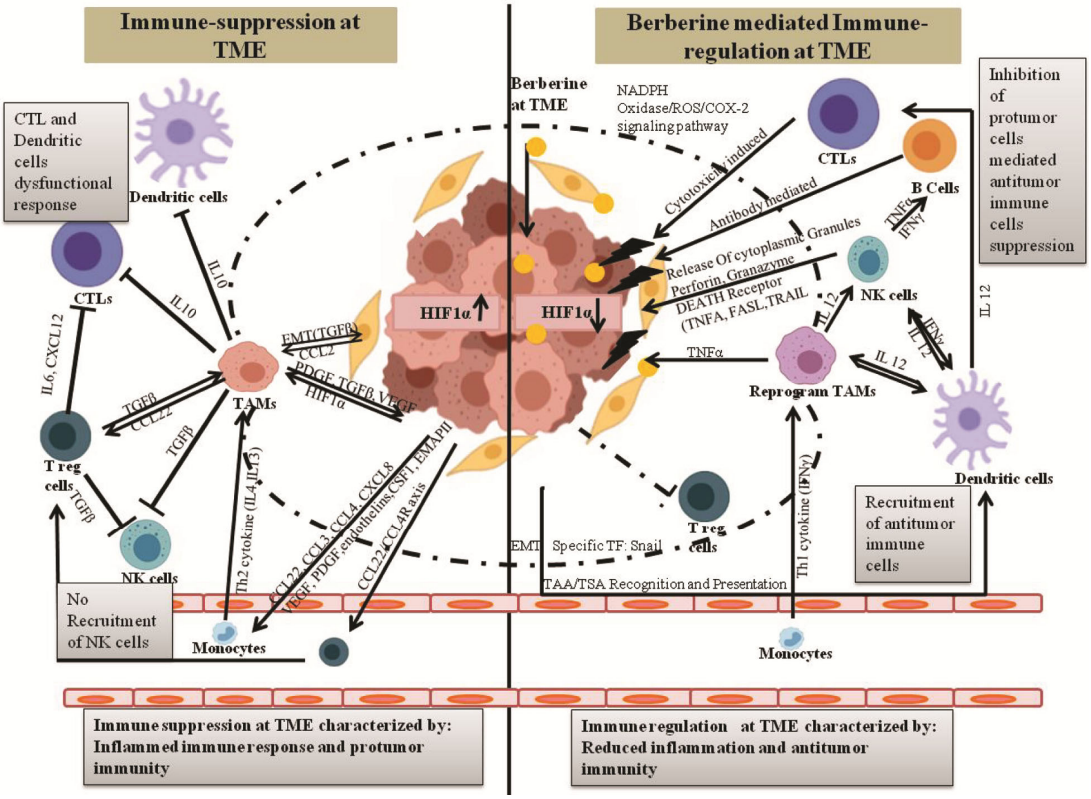

Fig. 4 — Berberine as an effective immunomodulator molecule at TME

tumor angiogenesis and metastasis in gastric cancer (AGS cells, MGC 803), triple negative breast cancer (TNBC cells), BRB inhibit IL-8 secretion *via* deactivation of phosphorylated expression of p38 MAPK, ERK1/2 and JNK signaling pathway, it also obstructs interaction of CXC motif chemokine receptor (CXCR1) and (CXCR2) *etc*. with their respective ligand on other pro-tumorigenic immune cells.BRB also down-regulate chemokine receptors expression of CCR6, CCR9, CXCR1, CXCR4 *etc*. in breast cancer (MCF-7) thus decreases tumorigenic interaction with in TME and drives it towards tumor regressive outcomes[47].

Berberine as an adjuvant molecule

BRB and TRAIL receptor (DR5) targeted therapy in breast cancer reveals enhanced TRAIL induced apoptosis in TRAIL-sensitive (MDA-MB-231) and resistant (MDA-MB-468) cancer cells this enhances therapeutic efficacy of anti-DR5 antibody when provided in combination with berberine[48]. BRB act as adjuvant to IDO1 inhibitor thus initiates tumor targeting through natural cells (NK cells) *via* activation of killer response against tumor growth. Its combination with other IDO1 related inhibitor can targetIDO1 expression, enzymatic activity for enhanced antitumor effectiveness. Further, on addition with IDO1 effect or modulator (mTOR, mammalian target of rapamycin; AhR, aryl hydrocarbon receptor; GCN2, general control over nonderepressible 2) it can also modulate immune suppression at TME by targeting IDO1 expression levels induced through related signaling pathways IFN-γ/JAK/STAT, PI3K/PKC, and NF-κB *etc*[49]. Thus, use of BRB as a novel adjuvant molecule frames a better approach for targeting and reprogramming immune cells along with tumor at TME for better therapeutic outcomes[50].

Targeting strategy of berberine against mutant p53

Targeting strategy against mutant p53 focus on apparent role of BRB in enhancing therapeutic efficacy against tumor recognized with immune suppression and chemoresistance. These targeting strategies may include: 1) mutant p53 regulation by microRNA 2) regulation of factors responsible for mutant p53 stability 3) post translational modification (PTM) of mutant p53 4) reactivation of wild type p53 expression (Fig. 5).

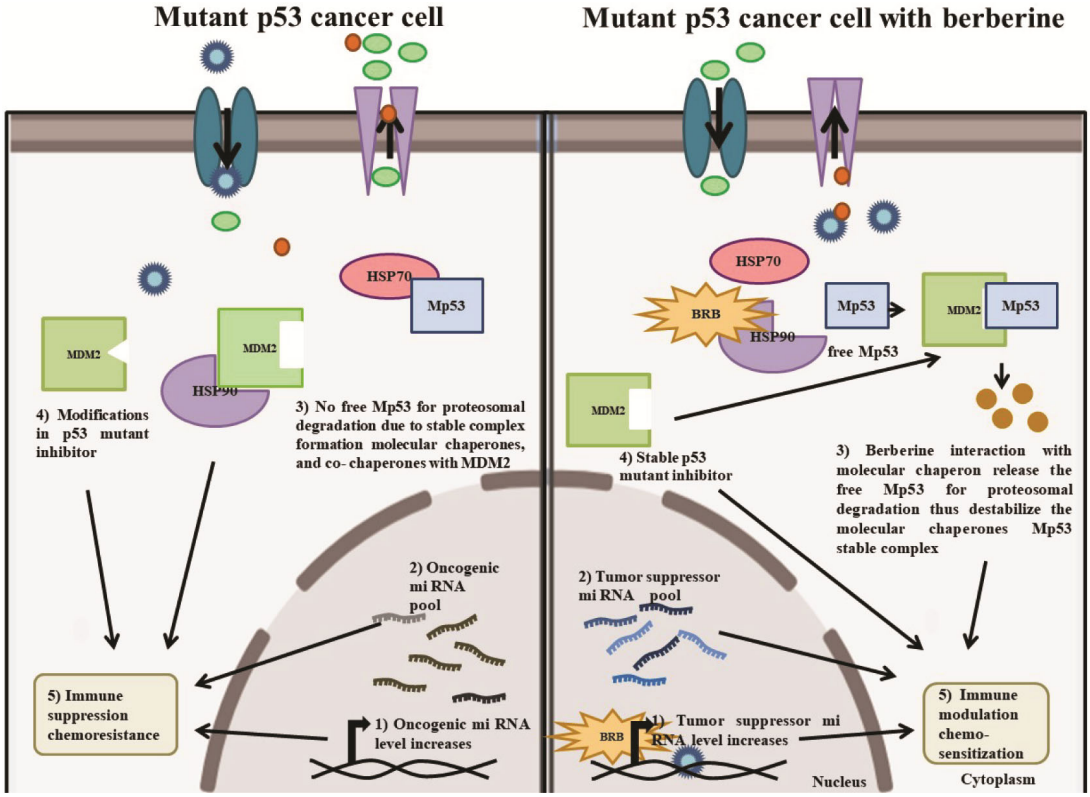

Fig. 5 — Schematic illustration of targeting strategy of berberine against mutant p53cancer cell

Regulation of mutant p53 by miRNA

MicroRNAs (miRNA) are non-coding RNAs ranging from 18-24 nucleotide pair specifically targeting messenger RNA (mRNA) by complementary base pairing. Presence of mutant p53 determines the expression ratio of tumor suppressor miRNA to oncogenic miRNA in cancer cells. Increased expression level mutant p53 tends to down regulate tumor suppressor miRNA expression level (miR-130b, miR-27a, miR-223) and up-regulates oncogenic miRNA (miR-155 miR-128-2) expression as reported in various cancers such as endometrial cancer, breast cancer, colon cancer *etc*[51].

BRB as inhibition inducer for oncogenic miRNA can target Mp53 mediated cancer stability and can provide a regulatory insight for tumor regression in various cancers. Down-regulation of miR-21 in BRB treated ovarian cancer is suggestive of its effectiveness against tumor growth, in multiple myelomathe mechanistic path of inhibition is depicted where BRB inhibits miRNA-21 by inhibiting IL-6 and STAT3 expressions thus, induces apoptosis. BRB in hepatocellular carcinoma alters the expression of MAT2A and MAT2B by up-regulating miR-21-3p expression, in colorectal cancer it obstructs Pin1-catenin–cyclin D1 signaling cascade by elevated expression of miR-296-5p, it also induces apoptosis with down-regulation of miR-429 expression. BRB is also known to up-regulate miR-203, in gastric cancer cells[51].

Regulation of p53 stability

Existence of mutant p53 within the cancer cells is a stability dependent phenomenon represented with insufficient working of ubiquitination mechanism or adapted PTM modifications in mutant p53 protein within cancer cells, which protects p53 mutant from being degraded therefore ensuring stable half-life of mutant p53 protein. This mutant expression further induces oncogenic shift by cellular ROS generation because of stable interaction of it with NADH quinone oxidoreductase 1 (NQO1)[52]. The possible reason behind deficient ubiquitination mechanism against mutant p53 in cancer cells can be its firm union with molecular chaperone present in cytoplasm such as HSP70. This principal chaperon molecule is known for its interactions with mutant p53 protein but to ensure enhanced stability it requires the presence of stabilizer molecule *i.e*., co-chaperone molecule such as HSP90. Presence of HSP90chaperone protein complexed with Mdm2 thus, direct inhibition of Mdm2 induced ubiquitination of mutant p53[52,53]. Other than this Pin1 expression is also known for transcriptional regulation enhancement for mutant p53 form[20,52]. Perhaps BRB and its related interaction studies with HSP90reveals the existence of BRB binding site on HSP90 chaperon. ATP binding domain on HSP90 is marked to be a molecular hot spot for BRB thus, binding of berberine can initiate reversal of stable complex that ensures enhanced half-life of mutant p53 within cancer cells. Synergistic effect of BRB was reported in combination with NVP-AUY922 (HSP-90 Inhibitor) against colorectal cancer, by CDK4 inhibition & miR-296-5p-induced repression of the Pin1–β-catenin–cyclin D1 signaling pathway[54].

Post-translational modifications of mutant p53

Post-translational modifications of p53 protein irrespective of its wild or mutant expression along with modifications in p53 mutant inhibitor (Mdm2/ MdmX) also contribute to its stability. DNA damage mediated phosphorylation of p53 at two different residues *i.e.,* serine 20 and threonine 18 prevent it from degradation *via* Mdm2 thus contributing to its activation and stabilization[55]. Constitutive phosphorylation by ERK also adds on to its stable foundation. Further acetylation induced mutant p53 gene stabilization is also known where activated expression of SIRT1is key destabilizing agent as its deacetylates mutant p53[56]. The arbitrary PTM within WT p53 and mutant p53 phenotype along with p53 mutant inhibitors under stress needed to be managed for least mutant p53 accumulation and activation, presence of BRB can definitely act accordingly destabilizing it within cancer cells.

Reactivation of the Wild-Type Function in mutp53

Targeting functional expression of mutant p53 at transcriptional and translational level can be subjugated to readjust the conformational state of mutant p53 from where it can be reactivated back to its wild type functional expression[57]. There are numerous pharmacological molecules known for this purpose referred as small molecules: PRIMA-1, MIRA-1 CP-31398 and SCH529074, RITA *etc*. probable binding of these molecules to mutant p53 ensure apoptotic cell death in mutant p53 cancer cells and promote wild p53 expression by shielding it from Mdm2 mediated degradation. Similar to it, binding of BRB to mutant p53 or p53 response element on targeted gene can provide a feasible direction and the new insight to manage p53 expression in cancer cells[57].

Conclusion

Recent advancement in cancer therapeutics aids to improve cancer malignancies by focusing on the risk factor associated with cancer resistance and immune suppression. Due to the limitation of conventional therapy, Plant derived phtochemicals such as berberine has evolved as potent anti-cancer agents. P53 being a chief regulatory mediator regulating numerous cancers related signaling pathway, targeting its mutated expression can redefine the new pathway towards functional wild type p53 expression in cancer cells. Versatile nature of BRB has enabled its use as a prime anti-cancer agent both as a chemosensitizer and as a immunomodulatory agent. The only issue that holds back its utilization is its poor solubility/ stability and bioavailability. These related issues can be managed by directing its utilization as a formulation at nano scale range. Nano formulation can either load or encapsulate BRB within nano-constructs to enhance its efficacy. The substantial role of BRB against mutant p53 expression marks new edges for novel and promising framework for cancer management therapies.

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Conflict of interest

All authors declare no conflict of interest.

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