ROS Modulatory Role of HDAC Inhibitors in Cancer Cells

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A Systemic Approach

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Abstract

Histone deacetylase (HDAC) inhibitors represent a novel class of therapeutic agents for cancer treatment. These enzymes play a pivotal regulatory role on chromatin epigenetics by deacetylation of histone proteins and lead to the production of reactive oxygen species (ROS) *via* activation of apoptotic pathway and autophagy. Surprisingly, the HDAC inhibitor-mediated ROS production in various cancer cells such as leukemia was found to be elevated by a combination of DNA damaging agents and proteasome inhibitors. In contrast, cancer cells possess proteins such as Trx and Trx reductase, as well as GSH peroxidase (Gpx) that help in preventing the oxidative stress of the cells by reducing the H_2O_2. Various bioinformatics tools can be utilized to understand the HDAC inhibitor-mediated differential gene expression data obtained by using the Affymetrix platform as well as the Illumina platform. Further, Gene Ontology (GO) and pathway analyses tools reveal pro-apoptotic gene expression signature and intrinsic apoptotic pathway during ROS generation. The differentially expressed genes were further subjected to Ingenuity Pathway Analysis (IPA) tool to study the associations of various molecular and cellular functions. ReMap (regulatory map of TF binding sites) analysis tool demonstrated the chromatin occupancy by proteins such as bromodomain-containing protein-4 (BRD4) and MYC proteins at their binding sites during HDAC inhibitor-treated cancer cells. Studies on HDAC inhibitor-mediated ROS generation and the tumor-suppressive effects can be better studied by using a combination of various molecular and bioinformatics methods that would help in better therapy against cancer.

Keywords

HDAC inhibitors · RNA seq · Microarray · Cancer · ROS · Proteomics · System biology

Abbreviations

4HNE	4-Hydroxynonenal
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ApoE−/− mice	Apolipoprotein E knockout mice

ATG7	Autophagy related 7
BAM	β-Barrel assembly machinery
BAX	BCL2-associated X, apoptosis regulator
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
Bid	BH3-interacting domain death agonist
Bim	Bcl-2-like protein 11
CD45	Lymphocyte common antigen
CD68	Cluster of differentiation 68
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CDKs	Cyclin-dependent kinases
CLL	Chronic lymphocytic leukemia
CTCL	Cutaneous T-cell lymphoma
CVD	Cardiovascular diseases
DR4	Death receptor 4
DR5	Death receptor 5
eNOS	Endothelial nitric oxide synthase
FAS	Fas cell surface death receptor
FOXO1	Forkhead box protein O1
GSH	Glutathione
H_2O_2	Hydrogen peroxide
HD	Huntington's disease
HDAC	Histone deacetylase
HDACi	HDAC inhibitor
HSP70	Heat shock protein 90
HSP90	Heat shock protein 70
HSPs	Heat shock proteins
LAQ-824	Dacinostat
LBH589	Panobinostat
MDM2	Mouse double minute 2 homolog
mHTT	Mutant huntingtin
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NOX1	NADPH oxidase 1
NOX4	NADPH oxidase 4
PCI24781	Abexinostat
PDH	Pyruvate dehydrogenase
PDKs	Pyruvate dehydrogenase kinases
PEITC	β-Phenylethyl isothiocyanate
PQC	Protein quality control
ROS	Reactive oxygen species
SAHA	Suberoylanilide hydroxamic acid
sHSPs	Small heat shock proteins
TNF-α	Tumor necrosis factor alpha

TRIAL	NF-related apoptosis-inducing ligand
UPS	Ubiquitin proteasome system
VEGF	Vascular endothelial growth factor
AKT	Protein kinase B
BSO	Buthionine sulfoximine
CRC	Colorectal cancer
GCL	γ-Glutamylcysteine ligase
Gpx	Glutathione peroxidase
Grxs	Glutaredoxins
GST	GSH-S-transferases
HIF-1α	Hypoxia-inducible factor-1α
IL-6	Interleukin-6
MAPK	Mitogen-activated protein kinase
mRNA	Messenger RNA
NBDHEX	Ethacraplatin, 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio) hexanol
NSCLC	Non-small cell lung carcinoma
PMX464	4-Benzothiazole-substituted quinol
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PX-12	1-Methylpropyl-2-imidazolyl disulfide
Ref-1	Redox factor-1
STAT3	Signal transducer and activator of transcription 3
TFAP2C	Transcription factor AP-2 gamma
Trx	Thioredoxin
TrxE	Trx reductase
TXNIP	Trx-interacting protein
ΔΨm	Mitochondrial membrane potential
Cav-1	Caveolin-1
COX2	Cyclooxygenase 2
CSCs	Cancer stem cells
Cu/ZnSOD	Zinc/copper superoxide dismutase
CXCL14	C-X-C motif chemokine 14
CYGB	Cytoglobin
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic reticulum
FoxA1	Forkhead box protein A1
FOXM1	Forkhead box M1
GR	Glutathione reductase
iNOS	Inducible nitric oxide synthase
JNKs	c-Jun N-terminal kinases
LDHA	Lactate dehydrogenase A
MMPs	Matrix metalloproteinases
MnSOD	Manganese superoxide dismutase
NOX5	NADPH oxidase
Nrf2	Nuclear factor erythroid 2-related factor 2

NSCLC	Non-small cell lung cancer
NUDT1	(Nucleoside diphosphate linked moiety X)-type motif 1
OCR	Oxygen consumption rate
PDH1	PDH kinase 1
Prx	Peroxiredoxins
SEPP1	Selenoprotein P1
SNAIl	Zinc finger protein SNAI1
SODs	Superoxide dismutases
TGF-β1	Transforming growth factor beta 1
UCP2	Mitochondrial uncoupling protein 2
uPA-	Urokinase-type plasminogen activator
VEGF	Vascular endothelial growth factor
AOS	Anti-oxidative stress
ICAT	Isotope-coded affinity tag
ISC	Iron-sulfur cluster
LC-MS/MS	Liquid chromatography tandem MS
SUF	Sulfur assimilation
ATM	Ataxia telangiectasia mutated
Bax	BCL2-associated X, apoptosis regulator
Bcl-2	B-cell lymphoma 2
Bid	BH3-interacting domain death agonist
Bmi1	Polycomb group ring finger protein 4
GSH	Glutathione
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate oxidase
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NRF2	Nuclear factor erythroid 2-related factor 2
SAHA	Suberanilohydroxamic acid (vorinostat)
SLC7A11	Cystine/glutamate antiporter xCT
SOD2	Superoxide dismutases 2
STAT3	Signal transducer and activator of transcription 3
TSA	Trichostatin A
VPA	Valproic acid
4SC-202	Domatinostat
BLCAP	Bladder cancer-associated protein
BRCA1	Breast cancer type 1 susceptibility protein
BRD4	Bromodomain-containing protein-4
BTG3	BTG anti-proliferation factor 3
CASP3	Caspase-3
CAT	Catalase
CDC2	Cyclin-dependent kinase 1
Cmap	Connectivity map
COL1A1	Collagen, type I, alpha 1
CSD	Cold shock domain
Ct	Threshold values

DUSP1	Dual-specificity phosphatase-1
Ect2	Epithelial cell transforming 2
EGLN1	Hypoxia-inducible factor prolyl hydroxylase 2
EPHX2	Epoxide hydrolase 2
ERMS	Embryonal rhabdomyosarcoma
FOXM1	Forkhead box protein M1
Foxo	Forkhead box transcription factors
G6PD	Glucose-6-phosphate dehydrogenase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GBM	Glioblastoma
GCLC	Glutamate-cysteine ligase catalytic subunit
GEP	Gene expression profiling
GO	Gene Ontology
GSS	Glutathione synthetase
HMOX1	Heme oxygenase 1
IDH1	Isocitrate dehydrogenase
Keap1	Kelch-like ECH-associated protein 1
LONP1	Lon protease homolog, mitochondrial precursor
MFA	Metabolic flux analysis
MM	Multiple myeloma
NAC	N-acetyl cysteine
NFE2L2	Nuclear factor, erythroid 2-like 2
NPC	Nasopharyngeal cancer
NQ01	NAD(P)H dehydrogenase [quinone] 1
NUDT1	Nudix hydrolase 1
PCI24781	Abexinostat
PON2	Paraoxonase 2
PR DX4	Peroxiredoxin
PSMMB5	Proteasome subunit beta type-5
RAD50	RAD50 double-strand break repair protein
ReMap	Regulatory map of TF binding sites
REST	Relative expression software tool
RNA-seq	RNA sequencing
RNF7	Ring finger protein 7
RNF7	Ring finger protein 7
Sepp1	Selenoprotein P
SGPP2	Sphingosine-1-phosphate phosphatase-2
SIRT1	Sirtuin 1
STAT1	Signal transducer and activator of transcription 1
STK24	Serine/threonine-protein kinase 24
TPx	Peroxiredoxins
TSA	Trichostatin A
TXNRD1	Thioredoxin reductase 1
TXNRD1	Thioredoxin reductase 1
YB1	Y-box binding protein1

Introduction

Histone deacetylases (HDACs) are the proteins that regulate the expression of several proteins involved in cancer. HDACs remove the acetyl groups and help in conversion of compacted chromatin to open confirmation. HDAC inhibitors were found to inhibit cancer initiation and progression. Several HDAC inhibitors are in phase I, II, and III clinical trials. These are promising anticancer drugs that modulate chromatin epigenetics and there by regulate gene expression. Studies have found that reversing epigenetic changes by targeting HDAC is a potent therapeutic strategy. Recent studies have identified the initiation, as well as the progression, of cancer that involves various types of epigenetic modifications. The HDAC inhibitors (HDACi) modulate several processes such as intrinsic apoptosis, extrinsic apoptosis, autophagy, cell cycle arrest, and tumor immunogenicity (Newbold et al. 2016). Treatment with HDAC inhibitors causes intrinsic apoptosis by upregulating the genes such as Bid and Bim and by decreasing Bcl-2 and Bcl-xL. Similarly, HDAC inhibitor-mediated extrinsic apoptosis involves the increased expression of DR4, FAS, and TRAIL (Bolden et al. 2013). HDACi induces G1/S phase cell cycle arrest by upregulating genes such as p21 and p15 and downregulating cyclin D1, cyclin E1, CDK4, and CDK6. Interestingly, HDAC inhibitors induce G2/M phase cell cycle arrest by the downregulation of cyclin A and cyclin B. These molecules also upregulate the FOXO1 gene, essential for autophagy (Lee et al. 2012) as well as decrease the natural killer cells' ligand expression (Murakami et al. 2008).

HDAC Inhibitors and ROS Production

The HDACi can induce apoptosis by upregulating the pro-apoptotic gene expression like Bam and Bax or by downregulating the anti-apoptotic genes like Bcl-2 and Bcl-xL selectively in cancer cells at the transcriptional level (Minucci and Pelicci 2006). They also elevate the reactive oxygen species (ROS) levels and activate various death receptors such as TRAIL, DR5, FAS, and TNF-α in cancer cells. Further, the high amount of cellular ROS affects the mitochondrial membrane potential and induces apoptosis *via* an intrinsic pathway (Rosato et al. 2003).

Petruccelli et al. (2011) reported that the SAHA induces double-strand breaks and enhances the cellular ROS levels in acute myeloid leukemia (AML) cancer cells, and also increases the caspase-3/7 activity as well as cell cycle arrest at the G2/M phase. PCI24781, a novel HDACi, induces apoptosis in a ROS-dependent manner and decreases the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-KB) expression (Sholler et al. 2013). LAQ-824, an HDACi, in combination with fludarabine promotes high intracellular ROS production and induces DNA damage and apoptosis in the leukemia cells. It also triggers pro-apoptotic protein expression and activates caspase-2 release from the mitochondria to the cytosol to initiate the apoptosis pathway (Rosato et al. 2008). Trichostatin A, an HDACi, downregulates the anti-apoptotic protein Bcl-2 and induces apoptosis in an oxidative stress-dependent manner in human cervical cancer cells.

HDACi induces the expression of Bid, a pro-apoptotic protein that disrupts the mitochondrial membrane potential and elevates the cellular ROS levels, thus inducing apoptosis (Ruefli et al. 2001). They also alter the antioxidant levels in cancer cells, by upregulating thioredoxin-binding protein-2 (TBP-2) which inhibits the activity of antioxidant protein thioredoxin (Trx) leading to enhanced ROS production at cytosol, thus inducing apoptosis (Butler et al. 2002). Apart from apoptosis induction, the HDACi can also induce cell cycle arrest by upregulating CDKN1A that codes p21WAF1/CIP (Richon et al. 2000), repressing the cyclin A and cyclin D gene expression. HDAC inhibitors also downregulate the vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) (Qian et al. 2006).

T315I mutation is the most common BCR/ABL mutation which is the main causative factor for the imatinib resistance. The combination of HDACi with ROS-inducing agents will enhance the efficacy in the treatment of cancer. Adaphostin (NSC680410) is a tyrosine kinase inhibitor that inhibits p210Bcr/abl expression, and activity in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) cancers (Chandra et al. 2006). It also elevates intracellular ROS levels, peroxide, superoxide, and glutathione and induces apoptosis along with the inhibition of electron transport in mitochondria of cancer cells (Nilsa Rivera De Valle et al. 2018). β-Phenylethyl isothiocyanate (PEITC), a redox modulatory agent, induces apoptosis and cell cycle arrest by downregulation of anti-apoptotic proteins like Bcl-2 and Bcl-xL in prostate cancer cells (Xiao et al. 2004). It also inhibits complex III in the electron transport chain inside the mitochondria and cytochrome p450 enzymes. Studies also indicated the ROS-enhancing activity of PEITC in cancer cells. Combination of a class of HDAC inhibitors such as MS-275, apicidin, and romidepsin killed nasopharyngeal cancer (NPC) cells with proteasome inhibitors such as bortezomib-induced apoptosis of nasopharyngeal cancer cells *via* reactive oxygen species (ROS) and caspase-dependent pathway (Hui and Chiang 2014). Recent studies have identified that the combination of HDACi with ROS-generating agents, proteasome inhibitors, methylation modulators, and DNA-damaging agents can be effective against cancers cells by inducing cell cycle arrest, apoptosis *via* high ROS production in mitochondria, histone hyperacetylation, and alteration in gene expression, ultimately killing the cancer cells (Miller et al. 2011) (Fig. 1).

HDAC Inhibitors and Protein Quality Control Systems

Eukaryotic cells possess efficient protein quality control (PQC) systems that include molecular chaperones, the ubiquitin proteasome system (UPS), and autophagy for the effective sensing of the proper folding and refolding of proteins. Interestingly, the PQC system modifies the epigenetic readout of HDAC inhibitor-treated cells (Michelle kulla et al. 2020). Further, PQCs help in the recognition of acetylation after HDAC inhibitor treatment and provide some more targets for effective treatment.

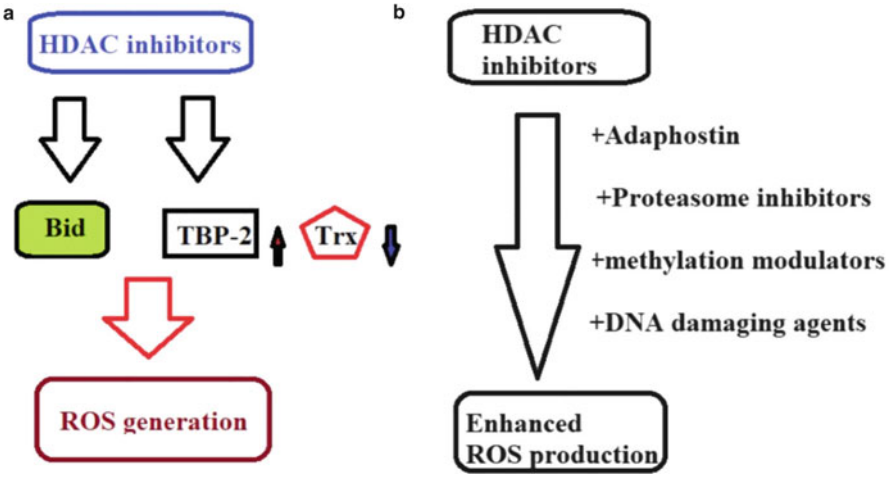

Fig. 1 HDAC inhibitor-mediated ROS generation in cancer cells. (**a**) In cancer cells, HDAC inhibitors induce Bid expression and enhance TBP-2 with a concomitant decrease in Trx protein level. (**b**) Combination therapy using HDAC inhibitors along with proteasome inhibitors (bortezomib), DNA-damaging agents (fludarabine), adaphostin, and methylation modulators induces enhanced ROS production

Molecular Chaperons

The heat shock proteins (HSPs) such as HSP90 family, HSP70 family, HSP60/GroEL family, and small heat shock proteins (sHSPs) act as vital molecular chaperons that regulate protein folding and were found to be affected by the acetylation (Jeng et al. 2015). HSP90 regulates protein folding and plays a major role in the activation of cell proliferation and signal transduction, thus promoting cancer, and interestingly, this protein directly binds to chromatin which makes this protein interplay in both epigenetics and molecular chaperones. The HSP90 activity was found to be impaired when it is treated with panobinostat (LBH589) or hyperacetylated by knockdown of histone deacetylase 6 (HDAC6) (Kovacs et al. 2005). HSP70 is involved in the protein folding and refolding, and high expression of HSP70 was associated with the methylation patterns of histone H3 in oral squamous cell carcinoma.

Ubiquitin Proteasome System

The proteasome recognizes the polyubiquitin chain of the proteins like metabolic enzymes, transcription factors, cyclins, and CDK inhibitors and degrades it by unfolding. These proteins play a vital role in cancer progression by proliferation and nutrient availability. The p53, a tumor suppressor gene, is reported to be degraded by the mouse double minute 2 homolog (MDM2) *via* poly-ubiquitination followed by proteasome degradation. This degradation can be prevented by inhibiting the proteasome activity of cancer cells where the p53 gene was mutated. Studies on the combination of bortezomib (proteasome inhibitor) and vorinostat or SAHA (HDAC inhibitor) induced synergic effects in inhibiting cancer cell proliferation (Johnson 2015).

Autophagy

Autophagy is acellular process that degrades cytoplasmic constituents misfolded, aggregated, and dysfunctional proteins when these proteins are very high amounts and can't be handled by the UPS and molecular chaperone PQC systems (Chun and Kim 2018). The inhibition of autophagy genes like Atg7 can induce apoptosis in colon and prostate cancer (Li et al. 2018). In imatinib-resistant CLL and colon cancer, it is observed that the HDACi induce autophagy, and the autophagy pathway induces back the pro-apoptotic and cytostatic effects of HDACi when used for combination treatment. Many HDACi such as Marbostat-100, trichostatin A, sodium butyrate, and YCW1 in a combination of various compounds have been reported to have significant effects on the PQC systems in various cancer cells like AML, breast cancer, colon cancer, and ovarian cancer (Noack et al. 2017).

Endogenous ROS Activation

Suberoylanilide hydroxamic acid (SAHA), an FDA-approved drug, was approved for cutaneous T-cell lymphoma. Recent studies with various prodrug and synthetic analog strategies have increased the selectivity against cancer cells. The abundant amount of endogenous ROS (H_2O_2) in cancer cells removes the OBP cap from the SAHA-OBP (novel SAHA prodrug), and thus an active SAHA drug is released in the cancer cells (Bhagat et al. 2018). The released SAHA reduces the HDAC6 protein levels and induces apoptosis by tubulin hyperacetylation. SAHA-OBP prodrug provides better efficacy that directs SAHA drug treatment toward cancer cells in terms of selectivity and stability.

Thioredoxin Role in ROS Regulation

In mammalian cells, the thioredoxin (Trx) system acts as an antioxidant mechanism by reducing the oxidized proteins. The Trx has two isoforms, namely, Trx1 (found in the cytosol) and Trx2 (found in mitochondria), and its family consists of various highly conserved thiol group proteins such as protein disulfide isomerases, glutaredoxins (Grxs), and quiescin sulfhydryl oxidase (Lee et al. 2013). It can maintain a reducing environment inside a cancer cell microenvironment by catalyzing the electron flux from NADPH to Trx in a two-step process. The N-terminal cysteine of Trx forms a disulfide bond with the substrate protein and then the Trx is oxidized and the substrate protein is reduced, and then the Trx is further reduced by Trx reductase (TrxR) using NADPH as an electron source to enhance the ROS scavenging activity.

In cancer cells, the components of the thioredoxin system such as Trx and Trx reductase were found to be overexpressed and Trx-interacting protein (TXNIP), which is under-expressed (Jia et al. 2019). The amount of Trx alters the ROS levels and can play a crucial role in cancer progression. It also involves the ROS

scavenging activity and gene expression of transcription factors such as redox factor-1 (Ref-1), hypoxia-inducible factor-1α (HIF-1α), nuclear factor kappa B (NF-κB), and tumor suppressor genes such as p53 and PTEN (Bassi and Stambolic 2013).

The high mRNA expression of Trx and TrxR is associated with various cancers such as breast, colorectal (CRC), oral, and prostate cancers and NSCLC, while the low expression of TXNIP mRNA levels is associated with breast, liver, and pancreatic cancers (Jia et al. 2019). The overexpression of Trx-1 promotes metastasis, and invasion in CRC shows resistance to docetaxel and cisplatin (Yamada et al. 1996), increases the transcriptional activity of forkhead box protein O1 (FOXO1) (Wang et al. 2015), and activates protein kinase B (Akt) (Li et al. 2012).

Tax inhibitors such as 4-benzothiazole-substituted quinol (PMX464), 1-methylpropyl-2-imidazolyl disulfide (PX-12), and suberoylanilide hydroxamic acid (SAHA) are reported to induce apoptosis and cell cycle arrest at the G2/M phase (You and Park 2017).

Glutathione Role in Antioxidation

Glutathione (GSH) is a tripeptide of glutamic acid (E), cysteine (C), and glycine (C) which is involved in the xenobiotic metabolism with the conjugation of GSH-S-transferases (GST) (Meister 1988). Nearly 90% of the GSH is present in the cytosol, followed by mitochondria and ER. In eukaryotic cells, GSH is present in both thiol-reduced (GSH) and disulfide-oxidized (GSSG) forms, and GSH is found to be 10–100-fold greater than the GSSG form that reacts with ROS and escapes apoptosis in cancer cells (Lushchak 2012). The elevated levels of GSH were found to be associated with cancer cell proliferation, invasion, and metastasis, and also the depleted levels of GSH in cancer cells seem to be more sensitive to treatment like chemotherapy (Marengo et al. 2010). GSH can act as a chemopreventive agent by detoxifying carcinogens and induction of antioxidant and anti-inflammatory responses as well as can also lead to carcinogenesis by evading apoptosis, drug resistance, and maintenance of redox levels in the cells.

The high expression of GSH-related enzymes, such as γ-glutamylcysteine ligase (GCL) and γ-glutamyl transpeptidase (GGT), is also associated with cancer cell growth. Buthionine sulfoximine (BSO), a GCL inhibitor, reduces the GSH levels and induces apoptosis and arrest cell cycle, and even it is also studied as a combination with various drugs for neuroblastoma (Marengo et al. 2011). The GST is overexpressed in cancer cells and directly binds to MAPK pathway kinases and alters its levels which leads to cancer drug resistance.

GSH peroxidase (Gpx) prevents the oxidative stress of the cells by reducing H_2O_2. Currently, eight GSH peroxidases (Gpx) are reported to be found in various parts of the body and are involved in cancer cell growth promotion by providing a strong antioxidant mechanism to the cells. Gpx1 is downregulated in breast cancer cells and its expression is regulated by the direct binding of the transcription factor AP-2 gamma (TFAP2C) to the Gpx1 promoter in the AP-2 regulatory region (Zhang

et al. 2020). The upregulation of Gpx8 is directly associated with EMT *via* regulation of IL-6 -STAT3, thus promoting stem cell nature in breast cancer. Gpx8 reduces the IL-6 production, leading to low cytokine production, thus resulting in the cancer phenotype.

Cancer cells overexpressed GSH-S-transferases (GST) that conjugate with a variety of anticancer drugs such as cisplatin, busulfan, and dichloroacetate, thereby showing drug resistance in multiple solid cancers. The direct inhibition of GST by ethacrynic acid, ethacraplatin, 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio) hexanol (NBDHEX), auranofin, and piperlongumine can help to overcome drug resistance (Allocati et al. 2018).

Reactive Oxygen Species: Types, Source, and Detoxification

Reactive oxygen species (ROS) are highly reactive, unstable, and partially reduced oxygen derivatives (ions, radicals, or molecules) produced as a by-product of cellular respiration and metabolic process. ROS is classified into free oxygen radicals and non-radicals and its detailed types were given in Table 1. Among all, superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$) are highly expressed and well-studied in various cancers (Liou and Storz 2010).

Table 1 Types of ROS observed in cells

Reactive oxygen species	Name	Symbol
Free oxygen radicals	Superoxide	$O_2^{\bullet -}$
	Hydroxyl radical	$^{\bullet}OH$
	Nitric oxide	$NO^{\bullet}$
	Organic radicals	$R^{\bullet}$
	Peroxyl radicals	$ROO^{\bullet}$
	Alkoxyl radicals	$RO^{\bullet}$
	Thiyl radicals	$RS^{\bullet}$
	Sulfonyl radicals	$ROS^{\bullet}$
	Thiyl-peroxyl radicals	$RSOO^{\bullet}$
	Disulfides	RSSR
Non-radical	Hydrogen peroxide	H_2O_2
	Singlet oxygen	$^1O_2$
	Ozone/trioxygen	O_3
	Organic hydroperoxides	ROOH
	Hypochlorite	HOCl
	Peroxynitrite	ONO^-
	Nitrosoperoxycarbonate anion	$O{=}NOOCO_2^-$
	Nitrocarbonate anion	$O_2NOCO_2^-$
	Dinitrogen dioxide	N_2O_2
	Nitronium	NO_2^+

During the cellular respiration, the superoxide ($O_2^{\bullet -}$) radicals are produced during the electron transport chain in the inner mitochondrial membrane and then released to the cytosol or mitochondrial matrix where they are scavenged by Cu/ZnSOD (zinc/copper superoxide dismutase) and MnSOD (manganese superoxide dismutase) into hydrogen peroxide (H_2O_2), respectively. Then H_2O_2 is reduced into H_2O by antioxidant enzymes such as catalase, glutathione peroxidase (Gpx), and peroxiredoxins (Prx).

ROS in Normal, Cancer, and Cancer Stem Cells

The intracellular ROS plays a major role in cell proliferation, differentiation, and vesicle trafficking, and elevated ROS levels lead to senescence and tumor formation. The high levels of ROS are scavenged by antioxidants such as superoxide dismutases (SODs), glutathione peroxidase (Gpx), glutathione reductase (GR), peroxiredoxin, and catalase present inside the cell (Forman et al. 2014). High ROS levels alter the lipid bilayer structure by inducing oxidative stress by peroxidation of fatty acids, affects protein function by oxidation of redox-reacting cysteine and/or tyrosine residues of signaling proteins, and induces mitochondrial DNA mutations of the gene that encodes for electron transport chain complexes. A result of high metabolic activity and mitochondrial dysfunction in cancer cells leads to high ROS production which can't be counteracted by the antioxidants.

In normal cells, glucose and glutamine were uptaken to produce ATP by anaerobic glycolysis followed by the Krebs cycle and oxidative phosphorylation in the mitochondria. Pyruvate produced at the end of glycolysis is catalyzed by pyruvate dehydrogenase (PDH) to produce acetyl-CoA to be used in the TCA cycle. Further, the ROS levels are maintained by antioxidants, and the mitochondrial membrane potential ($\Delta\Psi m$) and oxygen consumption rate (OCR) seem to be higher and with high production of ATP (Lleonart et al. 2018).

In cancer cells, ATP is produced from glycolysis rather than oxidative phosphorylation, thus resulting in a higher metabolic rate at mitochondria and endoplasmic reticulum (ER) (Dickinson and Chang 2011). Here pyruvate dehydrogenase (PDH) is inhibited by hypoxia-driven enzyme PDH kinase 1 (PDK1) and lactate dehydrogenase A (LDHA), thus converting pyruvate into lactate rather than acetyl-CoA (i.e., Warburg effect) which leads to the higher production rate of nucleic acids, amino acids, and fatty acids (Warburg 1956). Due to the mitochondrial membrane potential ($\Delta\Psi m$), the ROS levels were increased with a higher rate than the ROS scavengers, thereby leading to a malignant state.

In cancer stem cells (CSCs), the ATP production is similar to normal cells with high mitochondrial membrane potential ($\Delta\Psi m$) and oxygen consumption rate (OCR) with a high production of ATP (Song et al. 2015). The mediated ROS levels in the CSC show drug resistance with the cancer cell survival potency. It also upregulates FOXO1 (forkhead transcription factor), glutathione synthetases, and other antioxidant enzymes to maintain the intracellular ROS levels as a feedback loop process.

ROS Production

In normal somatic cells, ROS production helps in immune defense. In cancer cells, the production of ROS is increased due to environmental factors, such as smoking and UV, and intrinsic factors, such as increased metabolism; expression of various oncogenes such as c-Myc, Ras, and BRCA1; hypoxia condition, integrin activation; etc. (Yang et al. 2018).

ROS in Cancer and Role of Signaling Pathways

Superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) outcompetes the MnSOD levels and enables high cellular proliferation in cancer as a result of decreased expression of antioxidants. ROS mediates various signaling pathways such as MAPK/ERK1/2 pathway, PI3K/Akt pathway, and IKK/NF-κB pathways. In the MAPK pathway, H_2O_2 acts in the Cys118 residue of RAS and inhibits the GDP/GTP exchange or directly acts on ERK1/2 (downstream kinase of RAS), thus increasing the cell survival and growth (Steelman et al. 2008). ROS upregulates the mRNA of cyclin B2, cyclin D3, cyclin E1, and cyclin E2 that enables G1/S phase transition, thus leading to increased cell proliferation (Felty et al. 2015). ROS at high intracellular levels induce apoptosis by caspase activation *via* cytochrome c release from the mitochondria and also activate c-Jun N-terminal kinases (JNKs) that downregulate Bcl-2 and Bcl-XL (anti-apoptotic proteins) and upregulates Bax (apoptotic protein). Though low ROS levels promote cell survival and proliferation by regulating the cell cycle proteins (Dhanasekaran and Reddy 2017), elevated ROS levels activate NF-κB and nuclear factor (erythroid-derived 2)-like-2 factor (Nrf2) transcription factors that lead to apoptotic evasion, proliferation, and metastasis.

High ROS modulates the levels of β-catenin/Wnt, activator protein 1, HIF-1α (hypoxia-inducible factor-1 alpha), inflammatory cytokines, and growth factors leading to inflammation *via* activation of inducible nitric oxide synthase (iNOS) and downregulation of cyclooxygenase 2 (COX2) enzymes. ROS induces cytokine secretion *via* caspase1 activation and activator protein 1 protein levels (Forrester et al. 2018). ROS levels also play a major role in epithelial-mesenchymal transition (EMT) and migration by regulating uPA (urokinase-type plasminogen activator) *via* TGF-β1 upregulation and induction of hypoxia-mediated matrix metalloproteinases (MMPs), respectively. Moreover, high ROS levels upregulate C-X-C motif chemokine 14 (CXCL14) and enable cell motility during EMT (Liao et al. 2019). The ROS effects on gene expression in various cancers are given in Table 2.

Foxo Protein Signaling in ROS Generation in Lung Cancer

Foxo1, Foxo3, and Foxo4 are critically involved in cellular oxidative stress. FoxoM1 gene was found to be amplified in lung cancer (Leone et al. 2017). FoXo competes with TCF and binds at the TCF binding site of β-catenin and suppresses

Table 2 ROS and gene expression in various cancer cells

ROS	Cancer	Gene expression
H_2O_2	Lung	Bcl-2 ↓, pro-caspase-3↓, caspase-3 ↑, caspase-8 ↑
	Lung	Transglutaminases2 (TGase2, TGaseC) ↑
	NSCLC	Prevents Cav-1↓
	Lung	Heme oxygenase-1↑
	Lung	FoxA1↑, UCP2↑
	Breast	NF-κB↓
	Breast	VEGF↑, WNT1↑, CD44↑, E-cadherin↓
	Breast	CYGB↑, FOXM1↓, NOX5↓, NUDT1↓, SEPP1↓
	Breast	VEGF↓, MMP-2,9↓
	Gastric	MMPs↑ (1, 7, 14, 15, 17), β-catenin↑
O_2^-	Lung	Cav-1↑
	Lung	SNAIl↑, Slug↑, N-cadherin↑, vimentin↑, E-cadherin↓

↑ = Upregulation, ↓ = Downregulation

proliferation by inhibiting Wnt pathways. In addition, the FoXo M gene was found to be amplified in lung cancer.

ROS-Dependent Gene Expression in Cancer Cells

ROS is the reactive oxygen molecule involved in a variety of diseases such as cancer, and diabetes, neurodegeneration, etc. ROS is classified in free radical ROS with unpaired electrons or non-radical ROS such as H_2O_2 that can be converted to radical ROS. ROS is produced by the activation of enzymes cytochrome P450, NADPH oxidase, and cyclooxygenase and also by the activation of nonenzymatic enzymes involving mitochondrial-mediated respiratory chain. Excessive ROS can produce damage to DNA, proteins, and lipids and lead to an increase in ROS that results in cell death (Gorrini et al. 2013). In cells, the most important and widely studied antioxidant ROS scavengers include thioredoxin (Trx), nuclear factor erythroid 2-related factor2 (NRF2), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase.

ROS was found to be involved in initiation of cancer by activating various pathways including the ERK pathway as well as tumor angiogenesis (Fiaschi and Chiarugi 2012). Studies have shown ROS might induce certain oncogenes such as c-Myc and Ras leading to the stability of nuclear as well as mitochondrial stability. To counteract the changed ROS pattern, cancer cells alter the metabolic pathway that ultimately led to the tumor metastasis. RNA sequencing and DNA mutation status have identified the role of glutathione peroxidase (Gpx), peroxiredoxins (TPx), as well as several genes that are involved in dual-specificity phosphatase-1 (DUSP1), FoxM1, HMoX1, and superoxide dismutase (SOD). The correlation between oxidative gene signature and overall survival has identified gene expression changes involved in ROS metabolisms such as FoxM1, TXNRD1, DUSP1, EPHX2, NUDT1, RNF7, and SEPP1. DUSP1 is a dual-specificity phosphatase involved in

the inactivation of MAPK and acts as redox sensitizer (Kim et al. 2012). NUDT1 is a nudix hydrolase that functions in hydrolyzing the oxidized nucleotide leading to DNA damage (Waz et al. 2017). Ring finger protein 7 (RNF7) was found to be highly expressed in cancer cells which acts as a scavenger of ROS in cancer cells (Sun and Li 2012). EPHX2 is a cytosolic epoxide hydrolase involved in cancer metastasis and was found to be upregulated during oxidative stress (Bracalente et al. 2016). SEPP is a selenoprotein that possesses antioxidant properties and is a target of NRF2 family. Bioinformatic analysis by using STRING that predicts protein-protein interaction identified Gpx, SOD, and Trx pathways play an important role in dictating the cancer cell fate.

Anti-oxidative Stress (AOS) Genes

Anti-oxidative stress (AOS) scavenges ROS that arises during cellular metabolism. Gene set enrichment (GO), network, and pathway analysis have identified thioredoxin and glutathione pathways that are tightly associated with cancer. The key downstream targets of AOS are NRF2, NF-kappa B, FoxM1, etc. (Rotblat et al. 2014). Studies using the bio-profiling de GENE SRV tool reveals about particular gene signatures that enriched in various cancers and prediction of patient outcome. The key genes such as BTG3, CASP3, CDC2, G6PD, peroxiredoxin (PRDX4), NUDT1, PRDX4, HMOX1, GAPDH, PSMMB5, SEL, ECT2, EGLN1, LONP1 are involved in poor prognosis. The genes such as COL1A1, GAPDH, GCLC, GSS, NAD(P)H dehydrogenase quinone 1 (NQ01), RNF7, STK24, thioredoxin (TXN), and thioredoxin reductase 1 (TXNRD1) involved in prognosis in lung cancer. Genes that are important for good prognosis are PON2 and SIRT1 in breast cancer and NF-kB1 in lung cancer

HDAC Inhibition and ROS

HDAC inhibition results in tumor cell death by inducing reactive oxygen species (ROS). Interestingly, pretreatment with antioxidants results in the prevention of ROS as well as apoptosis. Cancer cells treated with HDAC inhibitors such as vorinostat and MS-275 induce ROS and caspase activation specifically in cancer cells but not in normal cells (Ungerstedt et al. 2005). Furthermore, treatment with non-hydroxamate NCH-51 resulted in enhancement in ROS level in leukemia cells and cytotoxicity by modulating the genes involved in antioxidation when compared with FDA-approved drug SAHA (Sanda et al. 2007). Surprisingly, SAHA-induced cytotoxicity was enhanced by using small interfering RNA against thioredoxin (Trx). A study by El-Naggar et al. (2019) found that class I HDAC inhibitors enhance Y-box binding protein1 (YB-1) acetylation and induce oxidative stress. Cancers usually inhibit ROS induction partly by the activation of nuclear factor erythroid 2-related factor 2 (NRF2). During oxidative stress, it was found that NRF2 protein gets stabilized

and gets dissociated from kelch-like ECH-associated protein 1 (Keap1). The possible ways of NRF2 activation include NRF2 activating mutations, Keap1 inactivating mutations, and oncogene activation.

HDAC Inhibitor and YB1 Relation in ROS Production

MS-275 HDAC inhibitors induce translational activation of NFE2L2 by YB1 (El-Naggar et al. 2019). HDAC inhibitors induce ROS in melanoma cells and acute myeloid leukemia (AML) (Petruccelli et al. 2011). MS-275 specifically targets HDAC-1 and HDAC-3 and induces ROS in leukemia (Rosato et al. 2003). MS-275 function increases YB1 acetylation (K81acetylation). Interestingly, mutation that converts K to E was observed in several cancers. YB1 was found to associate with nuclear protein filaments and bind to mRNA in the cytoplasm, thereby enhancing protein translational efficiency. It was found that YB1 protein is upregulated in breast cancer, colorectal cancer, sarcoma, etc. In breast cancers, it enhances the expression of genes that drives the metastasis such as SNAIL, ZEB2, and Twist1 (Evdokimova et al. 2009). In colorectal cancers (CRCs), YB1 regulates IGF1R. Y-box binding protein 1 is an RNA binding protein that binds to the 5′-untranslated region (5′-UTR) or 3′-untranslated region (3′-UTR) of various genes *via* its cold shock domain (CSD) (Eliseeva et al. 2011). Interestingly, in sarcomas, YB1 binds to 5′-UTR of the HIF1A gene and activates the HIF-1α mRNA synthesis (El-Naggar et al. 2015). Thus, YB1 regulation by HDAC inhibitor has a huge potential in controlling cancer cell metastasis.

HDAC Inhibitors and ROS-Mediated Effects on Cancer Cells

Studies have identified HDAC inhibitor-induced apoptosis is mediated by ROS-dependent mechanism. Hydroxamate pan-HDAC inhibitor LAQ-824 (40 nM) treatment at lower concentrations induces enhanced ROS generation and sustained DNA damage and upregulation of γ-H2AX foci. Interestingly, the apoptosis was not significant in those cells before treatment with LAQ-824 followed by fludarabine (0.4μM) results in enhanced apoptosis in leukemia cells *via a* drastic increase in γH2AX, phosphor ATM, ROS, and Bak expression (Rosato et al. 2008). The LAQ-824-induced ROS was found to be diminished by the treatment of ROS scavengers such as N-acetyl cysteine (NAC) or manganese (III) tetra kis-4-benzoic acid porphyrin (mn-TBAP). Interestingly, the treatment of HDAC inhibitor also involves an inhibitory effect on DNA repair genes *via* decreased expression on Ku86 and RAD50, BRCA1, RAD51, etc. Occurrence of this event also decreased the DNA binding activity of DNA repair proteins, activation of caspase-2, and release of histone H1.2 into the cytoplasm. Studies by Reczek and Chandel (2017) have indicated that oncogene, loss of tumor suppressor, enhanced metabolism, hypoxia, and low glucose play an important role in ROS in various cancers.

GBM vs. ROS

HDAC inhibitors have been shown to improve the outcomes by regulation of acetylation process in the preclinical setting. SAHA was found to be efficient in apoptosis induction in GBM cells (Premkumar et al. 2013). Furthermore, the combination of bortezomib and SAHA usage leads to effective therapy by enhancing the activation of Bax, Bak, cytochrome release (pro-apoptotic mitochondria injury), and γ-H2Ax foci with concomitant downregulation of Rad51 (Jane et al. 2011). HDAC inhibitors such as LBH589, LAQ 824, and trichostatin A combined with AEE 78 (inhibitor of MAPK, Akt) cause enhanced apoptosis in non-small cell lung cancer, ovarian cancer, and leukemia cells *via* ROS generation. This indicates HDACi have potential ROS inducers. Glioma cancer (GBM) isocitrate dehydrogenase IDH1 (R132H) causes resistance to HDAC inhibitors (Kim et al. 2019). Also, glioma with IDH mutations inhibits IDH catalytic activity and enhances hypoxia-inducible factor-1 α (HIF-α). IDH-R132H mutation led to HIF-1-α expression. Also, FAT1 (a typical cadherin 1), the upstream regulator of ROS, transcriptionally enhances HIF-α, and in turn, IDH R13H regulates FAT1 (Kumar et al. 2020). Studies have shown ROS production is regulated mainly through the hedgehog pathway.

ROS and reactive nitrogen are linked with the redox system in the tumor microenvironment. Chidamide (HDACi) treatment in GBM cancer cells leads to the upregulation of microRNA miR-338-5p located in intron 8 of apoptosis-associated tyrosine kinase (Lei et al. 2017). Studies have indicated that miR-338-5p mimics decrease U87, HS683, and MiR-338-5p. Researchers have found in gastrointestinal cancer cells, namely GI and GU, that the treatment of HDAC inhibitor induces the expression of Fas ligand (CD95L) and its receptor CD95 R (FAS receptor) leading to cancer cell death. Similarly, a combination of sorafenib and HDAC inhibitors kills GBM and medulloblastoma cells (Tang et al. 2012).

HDAC Inhibitors in Bladder Cancer

HDAC inhibitors (HDACi) romidepsin, trichostatin A, and vorinostat were found to be the most important chemotherapeutic agents for bladder cancer. Treatment with HDAC inhibitors resulted in the upregulation of protein expression of 2472 genes as well as downregulation of protein expression of 2049 proteins when compared to the untreated control cells. The bioinformatics analysis study has identified the involvement of these differentially expressed proteins in the regulation of cell cycle, cell death, free radical generation, and immune regulatory pathways. Proteomic analysis has also confirmed the role of HDAC inhibitors such as TSA and romidepsin on cell cycle, oxidative stress, apoptosis, etc.

Resistance Against HDAC Inhibitors in Gastric Cancer

Studies by Zhu et al. (2014) using a panel of gastric cancer cells have identified the important role of ribonuclease inhibitor known as RNH1 in driving the chemoresistance during HDAC inhibitor treatment (trichostatin A, SAHA). In addition, overexpression of the RNH1 gene in gastric cancer cells prevents ROS production and inhibits cancer cell apoptosis. This study has revealed the importance of ROS generation for effective apoptosis in cancer cells treated with HDAC inhibitors. The differential gene expression due to HDAC inhibitor was studied insensitively as well as resistant cell line using Affymetrix platform as well as Illumina platform. Studies have observed that seven gees such as ribonuclease inhibitor (RNH1), signal transducer and activator of transcription 1 (STAT1), C-X-C motif chemokine ligand (CXCL5), RAB40B (a member of Ras oncogene family), bladder cancer-associated protein (BLCAP), sphingosine-1-phosphate phosphatase-2 (SGPP2), and ELF

HDAC Inhibitors in Rhabdomyosarcoma

HDAC inhibitors such as TSA and SAHA induce myogenic differentiation and also inhibit tumor growth in embryonal rhabdomyosarcoma (ERMS), the most common soft tissue cancer in children, which is characterized by poor prognosis. Loss- or gain-of-function studies have identified the role of NOTCH1 and Ephrin B1 pathways that were regulated by HDACs to drive the tumor cell migration and inhibit the differentiation.

HDAC Inhibitors and Autophagy

Suberoylanilide hydroxamic acid (SAHA) stimulates autophagy in T-cell leukemia as evident by the accumulation of autophagic vacuoles and conversion of LC-III -I. SAHA upregulated Beclin-1 and Atg7 and promote Atg12-Atg5 (Li et al. 2010). Also, several HDAC inhibitors modulate apoptosis and autophagy in various cancer cells (Table 3).

Systemic Approaches

Rosenwasser et al. (2013) developed a bioinformatics tool named ROSMETER for the identification of transcriptomic imprints related to ROS (reactive oxygen species) in *Arabidopsis thaliana*. In ROSMETER, the transcriptome was given as query and the ROS signature profiles of biotic and abiotic stress-induced plants. This platform helps to identify the molecular-level mechanism of ROS in *A. thaliana*.

Table 3 HDACi-induced ROS and its mechanism in various cancers

Cancer	HDACi	Mechanism
Prostate	MHY4381 and SAHA	Induces apoptosis by increasing Bax/Bcl-2 ratio
Acute T-cell leukemia	SAHA	Depolarizes the mitochondrial membrane and induces cell death
Leukemia	SAHA	Cleaves Bid, induces mitochondrial damage and apoptosis
Leukemia	MS-275	Activates NF-κB, downregulates SOD2
Leukemia	LAQ-824	Modulates DNA integrity and induces DNA damage
Osteosarcoma	MS-275 (Entinostat)	Overexpression of NRF2
Acute myeloid leukemia	SAHA	Decreases GSH, induces DNA damage and apoptosis
Gastric cancer	TSA	Inhibits proliferation and induces apoptosis
Melanoma	SAHA	Suppresses SLC7A11 and induces apoptosis
Myeloma	SAHA and MS-275	Activates caspases, releases cytochrome c, and induces cellular death
Glioblastoma	SAHA	Activates caspases, induces autophagy, induces apoptosis in the late phase
Leukemia	SAHA	Increases BAX, downregulates survivin, induces apoptosis
Nasopharyngeal carcinoma	SAHA+ Bortezomib	Activates caspases, induces apoptosis via a non-mitochondrial pathway
Pancreatic cancer	VPA and TSA	Depolarizes the mitochondrial membrane, induces apoptosis and autophagy
Cervical cancer	TSA	Induces Bcl-2-mediated apoptosis and cell death
Breast cancer	TSA	Disrupts mitochondrial membrane and cell cycle arrest at G2/M phase and induces apoptosis
Hepatocellular carcinoma	VPA	Activates NADPH oxidase, overexpression of NRF2
Small cell lung carcinoma	VPA	Increases Bax/Bcl-2 ratio
B-cell lymphoma	VPA	Reduces ATM levels, induces apoptosis
Pancreatic cancer	VPA	Promotes the activation of p38, suppresses the activation of STAT3 and Bmi1

Cancer cells produce more antioxidants to scavenge ROS by the exploitation of anti-oxidative stress (AOS) response genes. Rotblat et al. (2013) examined the expression of 285 oxidative stress genes from 994 tumors and 353 normal tissues and found that 116 oxidative stress genes overexpressed in multiple types of cancers and under-expressed in normal cells. They also used Gene-set enrichment, Gene Ontology, network, and pathway analysis and found that the thioredoxin and glutathione pathway genes were correlated with cancer.

Yang et al. (2019) developed OxidizeME, a multiscale description of stress response induced by ROS in *E. coli*. It addresses major oxidative stress responses which include ROS-induced auxotrophy, nutrient-dependent sensitivity of growth,

ROS-specific differential gene expression, and coordinated expression of an iron-sulfur cluster (ISC) and sulfur assimilation (SUF) systems.

Posen et al. (2005) developed a precise tool photo-switch to analyze the ROS type and regulation at different doses in cell culture models. This tool enables us to exactly predict the ROS generation and regulation in cellular mechanisms in detail even in viable cells. They used hydroethidine as a detector and determined light-dependent generation of ROS in situ photogeneration of a nontoxic bacteriochlorophyll-based sensitizer in cell culture models. Even, it is helpful in the study of the ROS effects on various protein kinases involved in the cell cycle regulation and function.

Topf et al. (2015) used a quantitative proteomics approach to identify redox switches modulated by ROS. They performed a global proteomic analysis of nearly 2200 proteins in yeast redoxome, to map redox-active thiols influenced by ROS by various approaches that include isotope-coded affinity tag (ICAT) and state-of-the-art liquid chromatography tandem MS (LC-MS/MS). From this approach, they found that the high intracellular ROS levels cause mitochondrial dysfunction, which further leads to regulation of protein synthesis inside the cells.

System Biology Approach on Regulation of Molecular Pathways by HDAC Inhibitors in Cancer

Wittenburg et al. (2012) have employed Affymetrix canine v2.0 genechip in canine osteosarcoma cells treated with valproic acid (VPA) and have identified differential expression of various genes and the pathways by Meta Core software version 6.4 and have identified the involvement in cell cycle, cytoskeleton remodeling, the ubiquitin proteasome system, and oxidative phosphorylation. The significance of various genes involved in a particular pathway was confirmed by Fisher's exact test. Furthermore, the validation of microarray results was carried out by a real-time PCR study in which average threshold values (Ct) were used to confirm the gene expression changes using Relative Expression Software Tool (REST) v2.0.13 (Qiagen).

Computational Approach in Drug Discovery

It is well-known that Food and Drug Administration (FDA) drugs as well as the drug repurposing strategy reduce the cost and time to discover new drugs against a particular disease condition. Many of the recent day drugs can reverse the gene expression present in cancer cells, but researchers usually failed to consider the various functions and their dependencies' system level. In this regard, computational approaches that analyze the transcriptional data would help in the drug repurposing strategy and for effective drug discovery (Peyvandipour et al. 2018).

Potential Application of HDAC Inhibitors in Inducing Apoptosis

The normal fibroblast is transformed to a cancerous state by using various genes such as SV40-T antigens, hTERT, and mutant Ras (H-Ras ^{G12V}). The transformed fibroblast cells with oncogenes such as Ras (BJ LTSTERas) and normal (BJ) fibroblast cells that were treated with romidepsin (HDAC inhibitor) have indicated that HDAC inhibitors induce tumor-selective apoptosis. Furthermore, the differential expression of various genes was studied by using Affymetrix microarray analysis. In addition, Gene Ontology (GO) and pathway analyses have shown the involvement of apoptotic pathway. Further, IPA tool was employed to identify the impact of HDAC inhibitors on various molecular and cellular functions (Lamb et al. 2006).

Network analysis involving transcriptional profiling, metabolic flux analysis (MFA), and biochemical analysis was also employed to understand the enhanced glycolysis, tricarboxylic acid cycle (TCA), and use of glutamine in cancer cells. A recent clinical study has shown abexinostat (PCI24781) was known to inhibit class I and class II HDAC and induce apoptosis through the induction of reactive oxygen species (ROS) in B-cell lymphoma (Teodori et al. 2020). RNA sequencing studies in synovial sarcoma cells treated with quinostat that inhibit class I and class II HDACs have indicated an altered gene expression related to cell cycle arrest, neuronal differentiation, and reactive oxygen species generation (Laporte et al. 2017). In addition, microarray studies in thyroid cancer cells treated with PDX101 and LBH589 have shown common changes in cell cycle regulatory genes, DNA damage, as well as apoptosis-related genes. Also, pituitary tumor cell line AtT20 treated with SAHA has demonstrated downregulation of LXRα and upregulation of apoptosis-related genes (Lu et al. 2017). Interestingly, pancreatic ductal carcinoma cell lines treated with the HDAC inhibitor domatinostat (4SC-202) were subjected to RNA sequencing (RNA-seq) and ChIP sequencing, and the results have indicated an enhanced histone acetylation status, particularly H3K27ac in the promoter regions of the upregulated genes due to HDAC inhibitor treatment. Further studies by using ReMap (regulatory map of TF binding sites) analysis tool demonstrated the chromatin occupancy by bromodomain-containing protein-4 (BRD4) and MYC proteins at their binding sites (Mishra et al. 2017).

Gene expression profiling (GEP) and Gene Ontology enrichment analyses have indicated that 35 gene signatures are associated with the actin cytoskeleton and protein processing in the endoplasmic reticulum that are responsible for decreasing sensitivity of multiple myeloma (MM) cancer cells to HDACi (Mithraprabhu et al. 2013). Zhu et al. (2016) have studied global proteome and lysine acetylome, 3-plex SILAC-based quantitative proteomics technique, high-resolution LC-MS/MS, and bioinformatics analysis have identified a total of 1124 lysine acetylation sites in valproic acid and SAHA-treated AML cells. Surprisingly, the acetylome changes mediated by both the HDAC inhibitors were different from each other.

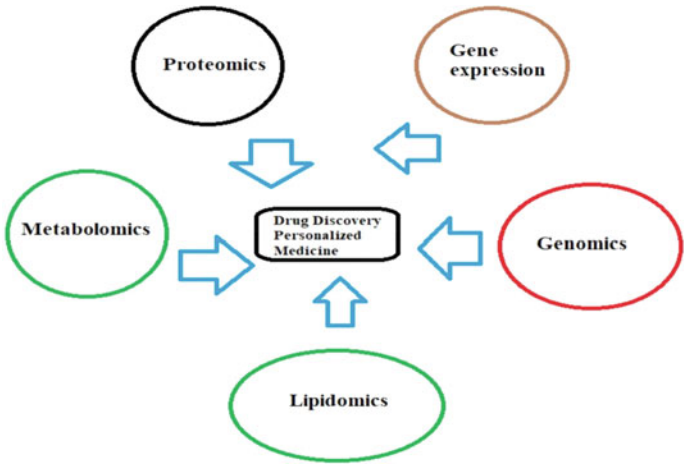

Fig. 2 Various approaches that are involved in personalized therapy and disease diagnosis

Conclusions and Future Directions

In summary, this chapter focuses on the role of HDAC inhibitors in the generation of ROS and apoptotic induction in cancer cells *via* intrinsic as well as extrinsic pathways. The ROS modulatory activity of HDAC inhibitors such as MS-275, vorinostat, and valproic acid in various cancers helps in effectively killing cytotoxicity. Furthermore, various proteins such as NF-kB and Foxo play a vital role in ROS generation. HDAC inhibitor-mediated ROS generation involves activation of Notch and Wnt pathways. In addition to epigenomics, mRNA expression profiling, microRNA expression study, and proteomics, metabolomics, and chemo-proteomics approaches have revealed the importance of the complete understanding of therapeutic response in cancer and various diseases. Studies using system biology and molecular biology approaches during HDAC inhibitor-mediated ROS will help in effective drug discovery, therapeutic response, and drug repurposing, as well as diagnostic aspects against cancer (Fig. 2).

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