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Chromatin remodeling by polyamines and polyamine analogs

Alice Pasini · Claudio M. Caldarera · Emanuele Giordano

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Abstract Natural polyamines are involved in many molecular processes, including maintenance of DNA structure and RNA processing and translation. Our aim here is to present an overview of the literature concerning the significance of polyamines in the modulation of chromatin arrangement and the transcriptional regulation of gene expression. The pleiotropic picture emerging from the published data highlights that these polycations take part in apparently diverging effects, possibly depending on the heterogeneous experimental settings described, and on a methodological approach aimed at the evaluation of the global levels of the histone chemical modifications. Since the relevant changes observed appear to be rather local and gene specific, investigating histone modifications at the level of specific gene promoters of interest is thus to be recommended for future studies. Furthermore, decoding the multiple regulatory mechanisms by which polyamines exert their influence on chromatin-modifier enzymes will reasonably require focus on selected individual polyamine-regulated genes. The evaluation of the many known chromatin-remodeling enzymes for their individual susceptibility to polyamines or polyamine derivatives will also be helpful: determining how they discriminate between the different enzyme isoforms is expected to be a fruitful line of research for drug discovery, e.g., in cancer prevention and therapy. Indeed, polyamine derivatives acting as epigenetic modulators appear to be molecules with great potential as antitumor drugs. All these novel polyamine-based pharmacologically active molecules are thus promising tools, both as a stand-alone strategy and in combination with other anticancer compounds.

Keywords Polyamines · Epigenetics · Chromatin · Histone acetyltransferases · Histone deacetylases · Histone demethylases

Abbreviations

ChIP	Chromatin immunoprecipitation
CpG	Cytosine preceding guanine
DFMO	α-Difluoromethylornithine
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
HDACi	Histone deacetylase inhibitor
LSD1	Lysine demethylase 1
ODC	Ornithine decarboxylase
PABA	Polyaminobenzamide derivatives
PAHA	Polyaminohydroxamic acid derivatives
PAOs	Polyamine oxidases
PcG	Polycomb group
PUT	Putrescine
SAHA	Suberoylanilide hydroxamic acid
SAM	S-adenosylmethionine
SC	Stem cell
SMO	Spermine oxidase
SPD	Spermidine
SPM	Spermine

A. Pasini · E. Giordano (✉)
Laboratory of Cellular and Molecular Engineering "S. Cavalcanti", Department of Electrical, Electronic and Information Engineering "G. Marconi" (DEI), University of Bologna, via Venezia 52, 47521 Cesena (FC), Italy
e-mail: emanuele.giordano@unibo.it

A. Pasini
e-mail: alice.pasini@unibo.it

C. M. Caldarera · E. Giordano
National Institute for Cardiovascular Research (INRC), University of Bologna, via Irnerio 48, 40126 Bologna (BO), Italy
e-mail: claudio.caldarera@unibo.it

TGase	Transglutaminase
TrxG	Thritorax group
TSGs	Tumor suppressor genes

Epigenetic regulation of chromatin layout: a brief outline

Gene expression is thoroughly regulated at the level of chromatin structure. Here, the accessibility of transcription factors at the gene promoter regions is modulated by a number of biochemical processes, such as the methylation of cytosine preceding guanine (CpG) in the DNA sequence or the post-translational chemical modifications of histone tails. Because of their heritable nature and their ability to determine a phenotype without affecting the genotype, these mechanisms shaping the active/repressed cell transcriptional layout are collectively referred to as "epigenetic" regulation.

The adduction of a methyl group at the CpG sites in a gene promoter region by DNA methyltransferases has been associated with a condensed chromatin structure and a resulting switch-off of the related gene expression (Bernstein et al. 2007). Although responsible for relevant physiological functions, such as silencing of chromosome X or stem cell (SC) differentiation, this process also appears to be involved in disease, e.g., cancer—where silencing of tumor suppressor genes (TSGs) may result (Esteller 2008; Herman and Baylin 2003). Methylated DNA recruits methyl-CpG binding proteins, which belong to large molecular complexes including histone deacetylases (HDACs), and corepressor factors that contribute to determine a compacted and transcriptionally silent organization of nucleosomes (Jones and Baylin 2002). HDACs catalyze the removal of acetyl groups from lysine residues of histone tails, increasing DNA affinity for histones, with a resulting more condensed chromatin structure responsible for downregulation of transcriptional activity. Histone acetyltransferases (HATs), on the other hand, account for the acetylation of histone tails and promote the activation of gene expression. Therefore, the balance between the activities of these two classes of enzymes dictates the fine regulation of chromatin architecture.

In addition to acetylation, histone tails are also targeted for other relevant covalent modifications, such as methylation and phosphorylation. Chromatin remodeling in either "open" or "closed" configuration results from the specific array—a real histone code—of the modified residues within histone tails (Bernstein et al. 2007). Whereas histone acetylation is typically associated with a transcriptionally active chromatin, the impact of histone methylation appears context dependent. Tri-methylation of lysine 27 in H3 histone tail (H3K27me3) indicates transcriptionally repressed chromatin. The same holds true for methylated H3K9. On the other hand, the promoter region of active genes appears enriched in methylated H3K4 and/or H3K36 (Shi et al. 2007). The concomitant presence of both active and repressive histone marks, H3K4me2/3 and H3K27me3, respectively, is also reported. These "bivalent" chromatin domains associate, e.g., to lineage-specific genes in SCs to keep them poised to be activated or repressed depending on the final cell fate (Bernstein et al. 2006; Fisher and Fisher 2011; Mikkelsen et al. 2007; Mohn and Schübeler 2009). The regulation of histone methylation is performed by enzymes collectively identified as histone methylases (or demethylases). Specific members of the Polycomb (PcG) protein complex act as methylases: PcG is involved either in normal development or in neoplastic transformation, and contributes to organize nucleosomes in a closed, transcriptionally repressed, chromatin. As an example, the EZH-EED PcG domain catalyzes the methylation of H3K27, a marker of transcriptionally repressed chromatin (Baylin and Ohm 2006). On the other hand, the Trithorax (TrxG) protein complex acts as an activator of gene transcription inducing increased levels of methylated H3K4. The opposite effect arises upon the action of the histone lysine demethylase 1 (LSD1) that catalyzes the demethylation of H3K4.

Histone methylation and acetylation thus represent important regulatory mechanisms of the epigenetic control of gene expression. Involved in various biological processes—including the physiological differentiation of SCs or the neoplastic cell degeneration—all these epigenetic changes are early events in the respective timeline. Understanding the epigenetic code, and possibly being able to address it, might offer significant tools for regenerative medicine and cancer therapy. In this light, several molecules interfering with the epigenetic mechanisms are under evaluation (Baylin 2005; Casero and Woster 2009; Grant and Dai 2012; Mai et al. 2005).

Polyamine interaction with DNA and chromatin-modifier enzymes

Polyamines are involved in many molecular processes, including regulation of DNA structure, as witnessed by a substantial body of literature (Igarashi and Kashiwagi 2010). Interestingly, it has been shown that polyamines and their analogs induce and stabilize the transition of B-DNA to Z conformation (Ohishi et al. 2008). This 3D modification is also promoted by DNA methylation at CpG islands (Temiz et al. 2012). Investigating the potential mechanistic relationship between these consistent evidences may increase our knowledge about polyamine influence on gene expression. It is worth noting that both the polyamine synthetic pathway and the DNA methylation mechanisms depend on S-adenosylmethionine (SAM), as a

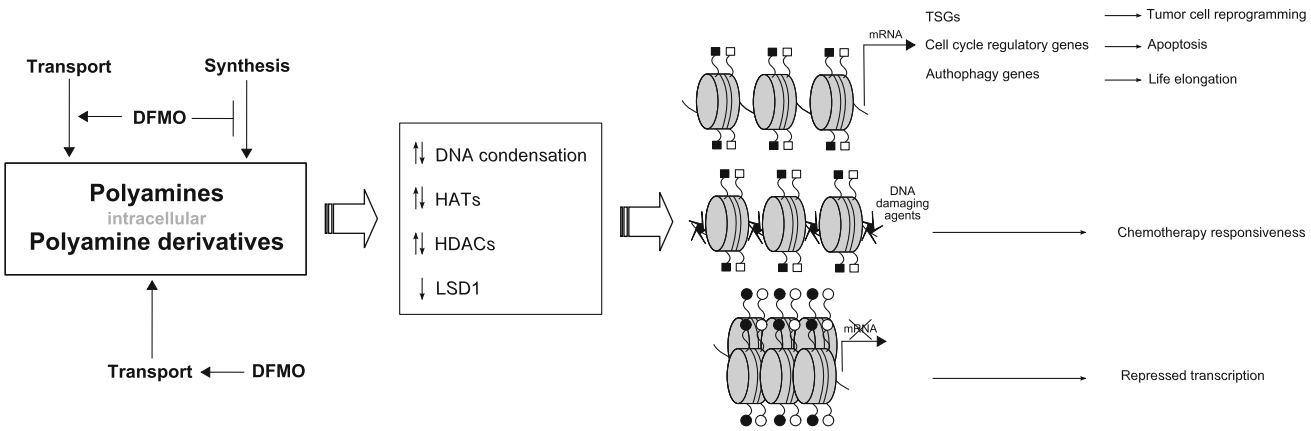

Fig. 1 Polyamine cellular content results from the balance of synthesis/degradation and intracellular import. α-difluoromethylornithine (DFMO)-induced natural polyamine depletion will increase polyamine, and polyamine derivative, uptake from the extracellular compartment. Polyamines and polyamine derivatives interact, directly or indirectly, with DNA and with chromatin-modifier enzymes, such as histone acetyltransferases (HATs), histone deacetylases (HDACs), histone demethylase 1 (LSD1), affecting the final relaxed or condensed chromatin structure. Gene transcription is allowed when nucleosomes are in an open configuration, presenting active histone marks (*square black* or *white*) such as H3K9ac, H3K4me2/3, whereas genes are silenced when nucleosome are in a close configuration with histone repressive marks (*circle black* or *white*) such as H3K9deacetylation, H3K27me3. An open chromatin structure also promotes the action of DNA damaging agents, with a resulting increased sensitivity of tumor cells to chemotherapy. Polyamine-induced reactivation of gene expression has been associated with: tumor suppressor genes (TSGs)—as *SFRPs* or *GATA4*—or cell cycle regulatory genes—as $p21^{waf1}$—that can reprogram transformed tumor cells or induce apoptosis; or to autophagy genes, in normal cells, producing life elongation

methionine backbone and a methyl donor, respectively. Polyamine biosynthesis impacts cellular folate requirements necessary to maintain SAM pools (Bistulfi et al. 2009). When the metabolism of polyamine synthesis is upregulated, a resulting DNA hypomethylation may follow and lead to a pathological gene expression profile. This has been reported, e.g., in autoimmune diseases where increased polyamines and DNA hypomethylation are frequently consistent events in disease development (Brooks 2012). This concept might also be extended to other diseases, including cancer, where an overexpression of enzymes in polyamine pathway has been observed.

As eukaryotic DNA is organized in a more complex structural architecture involving also histone proteins, our aim here is to present an overview of the literature (summarized in Table 1) concerning the significance of polyamines in the modulation of chromatin arrangement and the transcriptional regulation of gene expression (Matthews 1993). Just to cite some examples among the oldest reports, spermine (SPM) was described as an inducer of histone acetylation consistent with activation of gene expression in perfused heart (Caldarera et al. 1975), *Artemia* nuclei (Estepa and Pestaña 1981) or chromatin core extracts from rat liver cells (Dod et al. 1982). In agreement with these findings, NIH3T3 fibroblasts showed enhanced HAT activity and increased cellular proliferation after SPD administration (Desiderio et al. 1993). Upregulation of HAT enzymes was also shown in the same cells upon overexpression of ornithine decarboxylase (ODC), the first enzyme of polyamine metabolism that catalyzes the formation of putrescine (PUT) from ornithine. The resulting increase in histone acetylation was restored to the control level when these cells were depleted of polyamines by treatment with the suicide inhibitor of ODC, α-difluoromethylornithine (DFMO) (Hobbs and Gilmour 2000). In a following study, the same group showed the upregulation of HATs in the skin of transgenic mice overexpressing either ODC or ODC/RAS (Hobbs et al. 2002). DFMO treatment reverted the effect of ODC overexpression and restored histone acetylation to the level of control cells. In this study, however, a concomitant increase of HDAC activity was also reported, suggesting that polyamines did not influence the global chromatin structure but induced a local, gene-specific chromatin remodeling (Hobbs et al. 2002). This upregulation of HATs in normal skin and tumors from ODC/RAS transgenic mice was more extensively analyzed in a further study showing the concomitant upregulation of Tip60 and its variant Tip53. Both these enzymes belong to a multiprotein complex that is responsible for the acetylation of H4 histone tails (Hobbs et al. 2006). The concomitant upregulation of HAT and HDAC activities was reported in other studies using the same transgenic mouse models (Hobbs et al. 2003; Wei et al. 2007) pointing at the absence of a significant impact of polyamines on the global levels of histone acetylation. These findings support the hypothesis that the functional relevance of intracellular polyamines at the transcriptional level depends on selective promoter targeting.

Notwithstanding, other authors produced evidence that polyamines promote chromatin condensation associated

Table 1 Review of published data indicating a polyamine role in chromatin organization

Polyamine content	Biological effects	Experimental model	References
↑	Increased histone acetylation	Rat perfused heart—SPM administration	Caldarera et al. (1975)
	Increased histone acetylation	Artemia nuclei—polyamine (PUT, SPM, SPD) administration	Estepa and Pestaña (1981)
	Increased histone acetylation	Rat liver cells—SPM, SPD	Dod et al. (1982)
	HAT upregulation; increased cellular proliferation; no changes in polyamine acetylation level	Polyamine-depleted quiescent NIH3T3 mouse embryonic fibroblasts—SPD administration	Desiderio et al. (1993)
	HAT upregulation; HDAC downregulation; increased histone acetylation	NIH3T3 mouse embryonic fibroblasts—ODC overexpression	Hobbs and Gilmour (2000)
	HAT and HDAC upregulation; increased histone acetylation	Transgenic mouse skin—ODC overexpression; transgenic mouse skin and tumors—ODC/RAS overexpression	Hobbs et al. (2002)
	Histone polymerization influenced by TGase activity	In vitro proteins and DNA—polyamine (PUT, SPM, SPD) administration	Sato et al. (2003)
	HAT (Tip60 and Tip53) and HDAC upregulation	Transgenic mouse skin—ODC/K6 overexpression; transgenic mouse skin and tumors—ODC/RAS overexpression	Hobbs et al. (2003)
	HAT (Tip60 and Tip53) upregulation; Tip60 associated with E2F1 TF regulating gene expression	Transgenic mouse skin and tumors—ODC/RAS overexpression	Hobbs et al. (2006)
	HAT (Tip60 and Tip53) upregulation; p53 hyperacetylation	Transgenic mouse skin and tumors—ODC/RAS overexpression	Wei et al. (2007)
	Induced and stabilized Z-DNA conformation	DNA d(CGCGCG) crystallized—synthetic polyamine analogs	Ohishi et al. (2008)
	HATs (Iki3p, Sas3p) downregulation; decreased histone acetylation; autophagy; life elongation	Yeast, flies, worms, human cells—SPD administration	Eisenberg et al. (2009)
	Promoted DNA condensation	Plasmidic *e. coli* DNA—SPM, SPD administration	Sarkar et al. (2009)
	Increased DNA protection against protein digestion and damage	DU145 human prostatic carcinoma cells—SPD administration	Nayvelt et al. (2010)
	Chromatin condensation	In vitro DNA—SPM, SPD administration	Liu et al. (2011)
	HAT upregulation; HDAC1 upregulation; decreased histone acetylation	HDF human dermal fibroblasts—SPD administration	Park and Kim (2012)
↓	Increased sensitivity to chromatin digestion	U87 human glioblastoma cells—DFMO	Basu et al. (1992)
	Reduced affinity of acetylated polyamines for DNA	In vitro DNA—polyamine acetylation	Matthews (1993)
	Upregulation of gene expression	Yeast—GCN5 mutation, ARG3 upregulation, inhibition of polyamine synthesis	Pollard et al. (1999)
	Induced DNA structure favorable to transcription and replication; increased cellular proliferation	L1210 mouse lymphocytic leukemia cells—induced SPD acetylation	Wang et al. (1999)
	Easier extraction of acetylated histones	HCT116 human colon cancer cells—DFMO	Saunders and Verdin (2006)
	Increased histone H3 acetylation	Rat bone marrow mesenchymal stem cells—DFMO	Muscari et al. (2008)

The symbols ↑↓ indicate an increased or decreased polyamine content, respectively

HAT histone acetyltransferase, *HDAC* histone deacetylase, *TF* transcription factor, *PUT* putrescine, *SPM* spermine, *SPD* spermidine, *ODC* ornithine decarboxylase, *DFMO* α-difluoromethylornithine

with repression of gene transcription. Reduced levels of active spermidine (SPD) in L1210 cells were reported to induce a relaxed DNA structure, more favorable to transcription and replication, associated with an increase in cell growth (Wang et al. 1999). Pollard et al. showed that histone acetylation prevented chromatin condensation induced by polyamines in yeast. In their work, the mutation of the histone acetyltransferase GCN5 determined a decrease in histone acetylation and a consequent downregulation of specific target genes, such as *HO* and *SUC2*. Polyamine depletion induced by a limited availability of ornithine, which was converted to citrulline from the mitochondrial upregulated enzyme ARG3, restored *HO* and *SUC2* gene expression in GCN5 mutated cells (Pollard et al. 1999). Other studies also support the hypothesis that polyamines mediate chromatin condensation: glioblastoma U87 cells treated for 96 h with 1 mM DFMO present a chromatin highly sensitive to enzymatic digestion with DNAse I and MNase, suggesting that SPM and SPD catalyze nucleosome condensation, as reported by Basu et al. (1992); Muscari et al. tested the effect of polyamine depletion on bone marrow mesenchymal SCs induced to differentiate toward the cardiac phenotype: hyperacetylated H3 histone was scored at day 5 in cells treated with 1 mM DFMO for up to 9 days that also underwent cardiac commitment; inhibition of histone acetylation with anacardic acid abolished DFMO-induced differentiation, suggesting that polyamine depletion-dependent histone acetylation played a relevant role in driving the activation of early cardiac-regulatory genes (Muscari et al. 2008). Additional support for this view comes from the finding that SPM administration in aging yeast produces decreased histone acetylation as a consequence of HAT inhibition, whereas polyamine depletion leads to histone hyperacetylation. As a consequence, elongation of lifespan was reported when SPM was supplied to yeast, fly, worm and human cells. The mechanistic explanation was identified in the silencing of the majority of the genome with the exception of autophagy-relevant/pro-survival genes (Eisenberg et al. 2009).

Promotion of polyamine-dependent nucleosome condensation was also reported by Liu et al., who showed that SPM and SPD induced nucleosome core particle aggregation. This effect completely disappeared when H4 histone tails were either acetylated or deleted (Liu et al. 2011), confirming that acetylation of histone tails inhibits polyamine-induced chromatin condensation. The effect of polyamines on chromatin structure was also reported in the colon cancer cell line HCT116, where easier extraction of acetylated histones was reported upon polyamine depletion with 1 mM DFMO for 4 days (Saunders and Verdin 2006). In this study, also a decrease in HDAC inhibitor (HDACi)-induced apoptosis was scored in polyamine-depleted cells. The authors thus propose that changes in chromatin arrangement, induced by polyamine depletion, activate significant cell cycle checkpoints in tumor cells, which in turn undergo apoptosis when treated with HDACi (Saunders and Verdin 2006). These findings are also supported by Nayvelt et al., who showed that polyamines and polyamine analogs condense and stabilize DNA, and therefore reduce cell sensitivity to DNA damaging agents. Indeed, in this study, SPM and stereoisomers of methylated SPM protected DNA against enzymatic digestion and damage caused by hydroxyl radicals and by the topoisomerase-inhibitor etoposide in DU145 cells. As a further evidence, cells treated with DFMO showed a more pronounced DNA damage and were more prone to undergo apoptosis than cells supplemented with natural polyamines or their analogs (Nayvelt et al. 2010).

Polyamines appear to regulate HAT and HDAC expression also in human dermal fibroblasts. SPD supplementation determined a decreased HAT expression—although their activity was upregulated and also the increased expression of HDAC1 was scored. The balance of the two enzymatic activities resulted in a decreased global histone acetylation level. In this model, this picture finally associates with the inhibition of matrix metalloproteinase-2, a protein implicated in physiological degradation of extracellular matrix, tumor metastasis and invasion (Park and Kim 2012).

Acetylation of polyamines themselves is central to their physiological catabolism (Seiler 2004). Thus, a suggestive hypothesis about polyamine impact on chromatin structure is that, at least in part, they may recruit for this purpose the activity of enzymes also responsible for histone acetylation. Some authors support this view: Libby et al. identified two enzymes transferring an acetate moiety from acetyl-CoA to either of the histone tails and polyamines (Libby 1978). On the other hand, experimental evidences that histone and polyamine acetyltransferases depend on distinct enzymes have also been published (Desiderio 1992).

Polyamine-mediated chromatin remodeling appears also as a consequence of their role as an alternate substrate for transglutaminase (TGase), enzymes promoting histone polymerization through the covalent binding of glutamine and lysine. However, although PUT, and in some instances SPD, seem to inhibit protein polymerization by TGase, SPM was reported to induce increased histone polymerization (Sato et al. 2003).

The pleiotropic picture emerging from these published data about polyamine involvement in modulation of chromatin structure highlights that these polycations definitely influence its architecture, although the described diverging effects may depend on the distinct experimental settings explored. In addition, protocols measuring the global histone acetylation levels will unlikely show an unequivocal effect of polyamines. As the relevant changes observed

appear to be local and gene specific, the chromatin immunoprecipitation procedure, which investigates histone modifications at the level of specific gene promoters of interest, is thus to be recommended for future studies.

Some inconsistencies in the overall picture emerging from our analysis of the available literature may also result from the different strategies used to modulate the intracellular levels of polyamines. As an example, often the inhibitor of ODC, DFMO, is used to drastically reduce the intracellular amount of polyamines. DFMO binds to the active site of ODC and is decarboxylated, but stays covalently bound to the enzyme, therefore inducing its permanent loss of activity (Poulin et al. 1992). As a consequence, treating the cells with DFMO in vitro produces a suppression of polyamine synthesis. Inhibiting ODC using DFMO does not necessarily produce the opposite effects of administering exogenous polyamines. The effects of DFMO are highly dependent on the time of exposure, with prolonged treatment generally necessary to deplete SPD and especially SPM from the system. This ultimately leads to cell cycle arrest in the G1 phase: it is sufficient to produce a more relaxed chromatin state that may account for an increase in the global level of histone acetylation, independent of the lack of reported polyamine-related nucleosome stabilization (Meyskens and Gerner 1999). This is the case, e.g., in Basu et al. (1992) and Saunders and Verdin (2006), where 96 h with 1 mM DFMO determined the G1 block of most (>70 %) of the treated cancer cells.

When studying polyamine influence on chromatin structure, discrepancies regarding their reported role as repressors or inducers of gene expression might also depend on the experimental model, e.g., normal or cancer cells. These latter present a number of disregulated processes, including the epigenetic mechanisms of gene expression control, such as hypermethylated promoters or overexpressed HDACs; thus, they possibly do not represent the ideal testing benchmark.

A unifying explanation for these apparently conflicting reports is expected from the elucidation of how polyamines may mechanistically interact—directly or indirectly—with chromatin, including their (a) electronic affinity for DNA molecules, (b) modulating activity on histone modifier enzymes or (c) influence over other chromatin-remodeling factors (Fig. 1).

Synthetic polyamine-based modulators of chromatin structure for anticancer therapy

Although polyamines are involved in a number of (patho)physiological events (Brooks 2012; Fiori and Turecki 2008; Giordano et al. 2010; Govoni et al. 2010), the mainstream of inherent literature relates to cancer research (Gerner and Meyskens 2004; Casero and Marton 2007; Paz et al. 2011). Here, we present some polyamine-related agents for anticancer therapy, whose molecular mechanisms of action determine modifications of chromatin layout and interactions with the epigenetic regulation of gene expression.

HDAC inhibitors

Synthetic polyamine-based compounds were recently developed, with the aim of exploiting the upregulated polyamine membrane transport system, which is a common hallmark in cancer cells (Palmer and Wallace 2010), to funnel preferentially into neoplastic cells, the specific moieties bound to their aliphatic tail. Polyamine affinity for negatively charged DNA would target to chromatin the pharmacologically active component. Since another consistent feature in tumor cells is a condensed and transcriptionally repressed chromatin in the promoter region of TSGs, the interest of coupling a polyamine tail to an HDACi moiety clearly emerged as a pharmacological strategy for reactivation of gene expression. Several compounds have been designed to date following this principle: their general structure includes an aromatic cap group, an aliphatic linker chain and a metal-binding functional group, farther the polyamine side chain. A series of polyaminohydroxamic acid derivatives (PAHA) was first synthesized, with an SPM or SPD side chain and the hydroxamic acid moiety that is common to the stronger and commercially available HDACi, such as Trichostatin A (TSA) or suberoylanilide hydroxamic acid (SAHA) (Varghese et al. 2005). These compounds reduced class I and II HDAC activity in vitro, with the resulting increase of H3 and H4 histone acetylation in ML-1 mouse leukemia cells and in the colon cancer cell line HCT116; moreover, the cell cycle regulatory gene $p21^{WAF1}$ was reactivated, promoting the apoptosis of these neoplastic cells (Varghese et al. 2005, 2008). The ability of one among the tested compounds to induce acetylation of α-tubulin, but modest increase of $p21^{WAF1}$ expression and histone acetylation, suggests in this case a selective inhibition of HDAC6, indicated as responsible for metastatic invasion by cancer cells (Aldana-Masangkay and Sakamoto 2011). A series of polyaminobenzamide derivatives (PABA), including the HDACi MS-275 moiety, were next developed (Varghese et al. 2008). HCT116 cells, treated with the most active PABA compounds, showed decreased HDAC activity, hyperacetylated histones and increased levels of expressed $p21^{WAF1}$. The co-treatment with PABA and the demethylating agent decitabine produced a synergistic effect on the expression of the $p21^{WAF1}$ gene (Varghese et al. 2008). Some of the tested PABA derivatives induced apoptosis in

the breast cancer cell line MCF7 (Senanayake et al. 2009) and in one case apoptosis was associated with the induction of annexin A1 (Sharma et al. 2012), a reported effect of HDAC inhibition (Tabe et al. 2007). Three of them were reported to be selective inhibitors of the HDAC1 isoform (Sharma et al. 2012).

Experimental studies on polyamine transport system, through the ^{14}C-spermidine uptake competition assay, showed that PABA analogs were more effective substrates of the polyamine transporter than PAHA compounds, possibly due to differential charge distribution (Boncher et al. 2007; Varghese et al. 2008).

These new classes of polyamine derivatives with HDAC inhibitory activity represent promising molecules with potential as antitumor drugs, to be added to the current therapy. In addition, they may work as tools to identify, through their selective inhibition, the role of the defined HDAC isoforms.

HAT inhibitors

HATs and HDACs provide for the coordinated changes in the chromatin structure sketched above. This evidence suggests that also the inhibition of HATs would interfere with chromatin architecture, being potentially beneficial for cancer therapy, although limited progress in developing HAT inhibitors has to be acknowledged to date. An original inhibitor of HATs, with an SPD tail bound to a CoA group via a thioglycolic acid linkage, was recently synthesized. This compound was tested in a pool of cell lines to verify its effect, and the selectivity over neoplastic versus normal cells. The cancer cell specificity was expected from a reduced ability of normal cells to take up the compound compared to cancer cells, as tumors often display an upregulated polyamine transport system (Palmer and Wallace 2010). Arrest of DNA synthesis was scored in tumor cells, consistently with a delay in S-phase progression and inhibition of DNA repair. As expected, these biological events were consistent with the inhibition of histone acetylation. The effect of this SPD-CoA compound was also evaluated in conjunction with a variety of DNA damaging treatments, suggesting that it also sensitizes tumor cells to chemo- and radiotherapy (Bandyopadhyay et al. 2009).

LSD1 inhibitors

Demethylation of H3K4me1/2 by LSD1 is associated with transcriptional gene silencing. The evidence that LSD1 expression is consistent with an increased risk of cancer (Kahl et al. 2006; Scoumanne and Chen 2007) prompted exploration of the potential of specific LSD1 inhibitors in anticancer therapy. The amine oxidase domain of LSD1 shares 20 % sequence similarity with the FAD-dependent mono- and polyamine oxidases (PAOs), such as spermine oxidase (SMO) and N^1-acetylpolyamine oxidase; moreover, the catalytic domain of LSD1 showed 60 % similarity to amino acid sequences with SMO. Since compounds bearing guanidine groups were shown to inhibit both SMO and other PAOs, a pioneer series of (bis)guanidines and (bis)biguanides, previously synthesized as potential antitrypanosomal agents, was tested as inhibitors of LSD1 (Huang et al. 2007). Some compounds showed high, non-competitive, LSD1 inhibition at <2.5 μM and were able to induce the re-expression of aberrantly silenced TSGs in colon cancer cells in vitro, consistently with an increase of H3K4me2 and acetylH3K9 histone marks, and a decrease of the repressive H3K9me1 and H3K9me2 histone marks (Huang et al. 2007). The same effects on recombinant LSD1, as well as inhibition of human colon cancer HCT116 and RKO cell proliferation, were observed by a series of octamines and decamines (Huang et al. 2009). One among these compounds showed striking synergy in combination with the DNMT inhibitor 5-azacytidine in vivo against HCT116-derived tumors, without significant overall toxicity (Huang et al. 2009). This series of polyamine analogs was further expanded with (bis)urea and (bis)thiourea derivatives. Two of the most potent LSD1 inhibitors among these latter compounds were able to inhibit the growth of Calu-6 non-small cell lung carcinoma cells in vitro (Sharma et al. 2010). Similar results were also obtained in the estrogen receptor α-negative breast cancer cell line MDA-MB-231 treated with two LSD1 inhibitors: a polyaminobiguanide compound and the oligoamine PG-11144. The significant inhibition of cell growth was consistent with an increase in the expression of relevant target genes upon their administration (Zhu et al. 2012). The effect of PG-11144 was synergistically enhanced when colon cancer cell lines HCT116 and HC-29 were pretreated with DFMO: the intracellular polyamine depletion induced by DFMO stimulated the uptake of extracellular polyamines, including PG-11144 oligoamine. LSD1 was inhibited, the level of H3K4me2 increased, and the silenced TSGs *SFRP2* and *p16* genes were reexpressed, although the *SFRP2* promoter remained hypermethylated (Wu et al. 2012).

Since the epigenetic control via histone methylation/demethylation contributes to the complex regulation of gene expression, it provides a new target for the pharmacological modulation of relevant genes in cancer. Agents that modulate histone methylation and demethylation are under development by a number of groups and several lead compounds are presented in the literature. These new polyamine derivatives with LSD1 inhibitory activity are thus expected to represent promising molecules with potential as antitumor drugs.

Perspectives

The interaction of polyamines or polyamine-based analogs with nucleic acids and chromatin has received significant attention, although the molecular details of these processes are still under investigation. Decoding the multiple regulatory mechanisms by which polyamines exert their influence on chromatin-modifier enzymes to modulate gene expression will reasonably require focusing on individual polyamine-regulated genes. Moreover, the evaluation of the many known chromatin-remodeling enzymes for their individual susceptibility to polyamines or polyamine derivatives will be helpful: determining how they discriminate between the different isoforms of HDAC enzymes is expected to be a fruitful line of research for drug development, e.g., in cancer prevention and therapy. Gaining a deeper understanding about the competition for acetyl-group donors between the enzymes that acetylate histone proteins and those that acetylate polyamines would also be of value in this respect.

In addition to HATs and HDACs, histone demethylases were recently identified as target of an effective strategy for the treatment of cancer and other diseases. Therefore, polyamine derivatives acting as epigenetic modulators via their inhibition appear as molecules with potential as antitumor drugs. All these novel, polyamine-based pharmacologically active molecules are thus promising tools, both as a stand-alone strategy and in combination with other anticancer compounds.

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