[Frontiers in Bioscience 12, 528-535, January 1, 2007]

Acetylation in the regulation of metalloproteinase and tissue inhibitor of metalloproteinases gene expression

Ian M. Clark [1], Tracey E. Swingler [1] and David A. Young [2]

[1] School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom, [2] Musculoskeletal Research Group, School of Clinical Medical Sciences, Catherine Cookson Building, Medical School, Framlington Place, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, United Kingdom

TABLE OF CONTENTS

1. ABSTRACT

Together, the matrix metalloproteinases (MMPs) are capable of degrading every component of the extracellular matrix (ECM). Besides degradation of the ECM, MMPs release bioactive molecules from the matrix or cell surface and play important role in tissue repair after injury, development and in a number of pathologies including arthritis and cancer metastasis. Small molecules that inhibit a broad spectrum of metalloproteinases have not proved useful in the treatment of various diseases, probably due to the diverse roles of this large family of enzymes. An alternative therapeutic approach for a number of pathologies is to modulate the expression of specific metalloproteinase genes. Acetylation represents a recently identified covalent protein modification that is strongly implicated in transcriptional regulation. Histones were the first proteins demonstrated to show variable acetylation leading to gene activation. Subsequently, a large number of molecules including structural proteins, intracellular signaling molecules, nuclear membrane receptors and transcription factors were shown to be acetylated. Acetylation, like phosphorylation, is a reversible modification . Acetyl groups are added by a family of histone acetyl transferase enzymes (HATs) and are removed by histone deacetylases (HDACs). Inhibitors of HDACs (HDACi) have potent anti-proliferative and pro-apoptotic activities in cancer cells and may be used as cancer therapeutics. In this review, we examine the impact of changes in acetylation on the expression of the MMPs and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs). We discuss the suggestion that HDACi may act in a dual fashion: selectively decreasing cancer cell viability and reducing metastatic potential by decreasing stromal cell expression of specific metalloproteinases. Furthermore, we consider the possibility that selective HDACi have a potential as anti-inflammatory agents and in a range of degradative diseases such as arthritis.

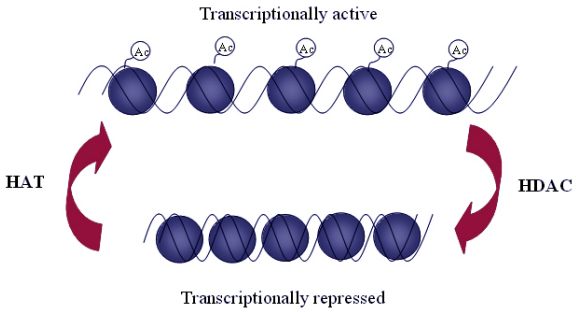

Figure 1. Chromatin structure and remodeling by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation of the N-terminal tails of histones leads to a loosening of the histone-DNA structure, allowing access of the transcriptional machinery. Furthermore, acetyl groups may associate with and recruit factors containing bromodomains.

2. INTRODUCTION

2.1. Metalloproteinase gene family

The matrix metalloproteinases (MMP) are a family of 23 enzymes in man. These enzymes were originally described as cleaving extracellular matrix (ECM) substrates with a predominant role in ECM homeostasis, but it is now clear that they have much wider functionality. Such functions include: opposing effects on angiogenesis via matrix degradation but also release of angiogenesis inhibitors (via digestion of e.g. plasminogen to generate angiostatin, and type XVIII collagen to generate endostatin); regulation of cell growth via release of cell surface-bound growth factors and receptors, release of growth factors sequestered in the ECM or integrin signalling; regulation of apoptosis via release of death or survival factors; alteration of cell motility by revealing cryptic matrix signals, or cleavage of adhesion molecules; effects on the immune system and host defense; degradation of chemokines (1).

Many MMPs are regulated at the level of transcription by a variety of growth factors, cytokines and chemokines. The promoter structure of several family members has been mapped to some extent, with a promoter proximal AP-1 site as the most obvious shared motif amongst inducible MMPs (2).

2.2. Acetylation and chromatin modifications

The role of chromatin modification, and in particular acetylation, is little researched in the metalloproteinase arena. The packaging of eukaryotic DNA into chromatin plays an important role in regulating gene expression. The DNA is wound round a histone octamer consisting of two molecules each of histones H2A, H2B, H3 and H4 to form a nucleosome. This unit is repeated at approximately 200bp intervals with histone H1 associating with the intervening DNA. Nucleosomes are generally repressive to transcription, hindering access of the transcriptional apparatus (3). However, two major mechanisms exist which modulate chromatin structure to allow transcriptional activity: firstly, ATP-dependent nucleosome remodellers such as the Swi/Snf complex (4,5); secondly, the enzymatic modification of histones, via acetylation, methylation and phosphorylation (6-8).

Acetylation by histone acetyltransferases (HATs) occurs on specific lysine residues on the N-terminal tails of histone H2A, H2B, H3 and H4. This neutralisation of positive charge leads to a loosening of the histone-DNA structure, allowing access of the transcriptional machinery; furthermore, the acetyl groups may associate with and recruit factors containing bromodomains (Figure 1) (3). Many transcriptional activators or coactivators have (or recruit) HAT activity, giving a mechanism whereby acetylation can be targeted to specific gene promoters (7,8). Conversely, histone deacetylases (HDACs) have also been characterised. Hypoacetylation of histones associates with transcriptional silence, and several transcriptional repressors and co-repressors have been identified which have (or recruit) HDAC activity (9-11). Over 80 non-histone substrates of HATs have also been described, including ~40 sequence-specific transcription factors (e.g. p53, NF-κB, STAT-3 and c-jun (12-16)), and this diversity suggests acetylation represents a biological modification rivaling phosphorylation (8).

2.3. Histone deacetylases and inhibitors

There are two families of HDACs, the NAD^+-dependent, so-called SIR2 family (sometimes called class III HDACs) and the classical HDAC family. The classical HDACs can be split into three classes (I, II and IV) based on phylogeny (17). Class I HDACs (HDAC1, 2, 3 and 8) are related to yeast RPD3, and class II HDACs (HDAC4, 5, 6, 7, 9 and 10) are more closely related to yeast HDA1 (9). HDAC11 alone represents class IV and HDAC11-related proteins have been described in all eukaryotic organisms other than fungi (17). All classes of the classical HDACs exist in bacteria which lack histone-related proteins, suggesting that the ability to deacetylate histones is not necessarily the main function of these enzymes (17). However, all 11 mammalian HDACs have histone deacetylase activity and act at least in part as, or associate with, transcriptional repressors although they also appear to have additional functions. Trichostatin A (TSA) and sodium butyrate (NaBy), are HDAC inhibitors (HDACi) (18,19) with a broad spectrum of activity against class I and II HDACs, but not the SIR2 family. Addition of these reagents to cells should therefore block histone deacetylation and result in increased acetylation of histones on susceptible genes. The prediction would be that this would lead to an increase in gene expression, however, there are many instances of HDACi acting as repressors of gene expression (20-24) and data from microarray studies indicate that an approximately equal number of genes are activated or repressed following HDACi treatment (25-27).

HDACi have potent anti-proliferative and pro-apoptotic activities in cancer cells and this has led to the development of specific inhibitors for cancer chemotherapy. Such compounds are currently in both preclinical development and clinical trials (28). For example, MS-275, a synthetic benzamide-containing HDACi is currently undergoing a phase II study in patients

with refractory solid tumours and lymphomas; remarkably, considering the possible global gene regulation modulatory effects of these compounds the initial trial of MS-275 showed no dose-limiting toxicity effects, an important observation should more specific HDACi be eventually evaluated for treatment in metalloproteinase-mediated conditions (29). Interestingly, the anti-epileptic drug, valproic acid, inhibits HDACs, preferentially those in class I. Whilst the anti-epileptic action of valproic acid does not appear to be mediated by its ability to inhibit HDACs, the lack of major toxicity during its long term use demonstrates that inhibition of HDACs (at least class I HDACs) is therapeutically possible (30).

3. ACETYLATION AND ITS IMPACT UPON METALLOPROTEINASE EXPRESSION

3.1. Chromatin modifications and MMP expression

The first report of acetylation impacting upon *MMP* gene expression was by Pender et al. (31) working with human fetal mucosal mesenchymal cells. In this paper, NaBy and TSA were shown to enhance IL-1β or TNFα induction of MMP-3 and to repress IL-1β or TNFα induction of MMP-1 and MMP-9 both at the mRNA and protein level. No effect of these HDACi was seen on unstimulated levels of any MMP examined, and no effect was seen on MMP-2 or TIMP-1, neither of which was induced by IL-1β or TNFα. From these data, it is likely that the effect of HDACi is on the signaling pathways induced by these proinflammatory cytokines, rather than on the *MMP* genes themselves, though this is currently unknown. It is interesting that the same HDACi can lead to enhancement or repression of an induced MMP (compare MMP-3 to MMP-1 and -9 above): this might suggest that there are differences in the pathways by which IL-1 or TNFα induce MMP-3 compared to MMP-1 and -9 or that there are subtle differences in the impact of HDACi on e.g. NFκB that are gene specific (see below).

Ailenberg and Silverman (32,33) then described that TSA (across a 6-50ng/ml dose range) could repress *MMP2* gene expression at the mRNA level and also the activity of the enzyme in NIH 3T3 cells. Since TSA is a hydroxamate, a moiety that can chelate zinc, they also show that TSA does not directly inhibit MMP-2 activity in the assay used. In this cell line, TSA did not alter expression of either *MT1MMP* or *TIMP2*, factors known to be involved in proMMP-2 activation. Interestingly, the human fibrosarcoma cell line HT1080 is much less sensitive to TSA with little effect on *MMP2* expression even at 200ng/ml. In line with the dogma, these authors also found that TSA induced apoptosis in the transformed HT1080 line, but not in the 3T3 cells. They therefore suggest that TSA, and other HDACi in development as cancer therapeutics may act in a 'two-pronged' way: selectively decreasing cancer cell viability and reducing metastatic potential by decreasing stromal cell expression of MMP-2.

Similarly, Kaneko et al., (34) demonstrate both reduced invasion through Matrigel, and reduced MMP expression and activity, in response to NaBy. In human liver cancer cell lines, treatment with NaBy (2mM) reduced activity of MMP-2 and MMP-9 and expression of *MMP1*. The expression of *TIMP1* and *TIMP2* was unaffected. Since the effect of NaBy on MMP levels was similar to that of interferon-α, the authors speculate on cross-talk or commonality between the signalling pathways activated by these factors.

Interestingly, valproic acid was shown to be proapoptotic in LCCaP human prostate cancer cells with concomitant upregulation of *TIMP3*. These authors also report a similar induction of *TIMP3* by TSA (35).

These papers do not dissect in detail the mechanism(s) by which HDACi impact upon *MMP* gene expression in terms of the HDAC involved, nor in terms of the number of MMPs affected by an HDACi.

Martens et al (36) consider the induction of the *MMP1* gene in T98G human glioblastoma cells by a combination of serum and phorbol ester (phorbol-12-myristate 13-acetate, abbreviated PMA or TPA). This combination gives a potent induction of *MMP1* expression at the mRNA level between 90 min and 2 hours with a maximal induction at 4-6 hours. These authors used chromatin immunoprecipitation (ChIP) to identify factors recruited to the *MMP1* promoter in this system and examine the kinetics of histone modifications occurring with induction of the gene. Acetylation *per se* is insufficient to induce *MMP1* expression in these cells since TSA had no effect on *MMP1* expression despite an increase in local H3 acetylation at the MMP-1 promoter. It appears that upon activation with serum and TPA, c-Jun, c-Fos, TBP, RNAPII and SET9 assemble on the *MMP1* promoter and histones at this location are dimethylated (and eventually trimethylated). p300 and RSK2 are then recruited which correlates with an increase in acetylation and phosphorylation of histones at the *MMP1* promoter. Finally, Swi/Snf, an ATP-dependent nucleosome remodeling complex is recruiting allowing initiation of transcription.

In a similarly detailed dissection of events at the promoter, Ma et al (37) examined PMA induction of the *MMP9* gene in HeLa cells. Increased *MMP9* mRNA is apparent by 2 hours of PMA treatment and becomes maximal at 6 hours. An NFκB site, two AP1 sites and an Sp1 site in the *MMP9* promoter are all involved in induction. Micrococcal nuclease digestion demonstrates that the *MMP9* promoter is in a regular nucleosomal array, and chromatin remodeling is necessary for *MMP9* transcription. This latter is elegantly proven by using a Brg1-deficient cell line (SW-13 cells); Brg1 is the ATPase subunit of the Swi/Snf complex. In these cells, *MMP9* is not expressed on PMA stimulation, but transfection with Brg1 (but not an ATPase-null mutant) rescued responsiveness of *MMP9* expression to PMA. ChIP experiments demonstrated recruitment of AP1 factors, NFκB factors and Sp1 at appropriate time points, and modifications in histone acetylation, phosphorylation and methylation. MTA, a specific protein methyltransferase inhibitor, suppressed PMA-induced *MMP9* expression,

whilst TSA enhances PMA-induced *MMP9* expression. These authors also demonstrated that both HDAC1- and HDAC3-containing complexes occupy the *MMP9* promoter in unstimulated HeLa cells, but that these are removed upon stimulation with PMA. This was reinforced by the repressive effects of HDAC1 or HDAC3 (but not HDAC2 or HDAC4) on PMA-induced *MMP9*.

Both of these papers (36,37) demonstrate a coordinated cascade of cell signaling, histone modifications, nucleosome remodeling and recruitment of transcription factors. This may be cell type, species, or stimulus-specific.

Young et al (38) show that both TSA and NaBy block IL-1/OSM-induced cartilage resorption in an explant model in a dose-dependent manner at the level of both proteoglycan and collagen release. This was accompanied by a reduction in activity and activation of procollagenases in the conditioned medium and a similar reduction in gelatinase activity. In cultured primary chondrocytes, TSA and NaBy repressed the IL-1/OSM-induction of collagenolytic MMPs (MMP-1 and -13) and aggrecanases (ADAMTS-4, ADAMTS-5 and ADAMTS-9). In SW1353 chondrosarcoma cells, the majority of metalloproteinase genes that were robustly induced by the IL-1/OSM combination, were then repressed by the HDACi, these were *MMP1*, *MMP3*, *MMP7*, *MMP8*, *MMP10*, *MMP12*, *MMP13* and *ADAMTS9*. The basal expression of several metalloproteinase genes was also induced by HDACi, these were *MMP17*, *MMP23*, *MMP28*, *ADAMTS15* and *ADAMTS17*, as well as the inhibitor *TIMP2*. These data suggest that HDACi impact upon IL-1/OSM signaling to repress induced levels of metalloproteinases and that the basal expression of a number of metalloproteinases are responsive to HDACi. There is no overlap between these groups, which may point to mechanisms/pathways by which groups of genes are coexpressed in these enzyme families.

Young et al (39) detail the differential effects of HDACi on the *TIMP1* gene dependent on the stimulus used to induce the gene. HDACi enhance PMA-induced *TIMP1* expression but repress TGFβ-induced *TIMP1* expression. Interestingly, the dose-response curves for these two effects are different with e.g. >500ng/ml TSA required for the maximal enhancement of PMA-stimulated *TIMP1* but only 5ng/ml TSA required for the repression of TGFβ-induced *TIMP1*. This strongly suggests that different HDACs are the target of HDACi in each case, and this is reinforced by the fact that valproic acid also enhances PMA-induced *TIMP1*, but does not block TGFβ-induced *TIMP1*. Furthermore, the effect of HDACi on the endogenous *TIMP1* gene can be reiterated at the level of transient transfection of promoter-reporter constructs. This perhaps suggests that their effects are more likely mediated by acetylation of signaling molecules or transcription factors rather than histones.

3.2. Transcription factor and signaling pathway acetylation and MMP expression

At the simplest level, acetylation of histones at a gene promoter may increase access to cis-acting sequences by their cognate transcription factors. However, acetylation and deacetylation of signaling pathway components and transcription factors themselves makes the situation complex (Figure 2). This complexity is undoubtedly increased by cell specific differences in some of these events.

3.2.1. AP-1

Many inducible MMP genes (e.g. *MMP1*, *MMP3*, *MMP7*, *MMP9*, *MMP10*, *MMP12*, *MMP13* (2) contain promoter proximal AP1 sites that are key features of their inducibility. In order for c-Jun to activate target gene transcription, it requires phosphorylation by the Jun-N-terminal kinase (JNK) at serines 63/73 and threonines 91/93. An 'activation by de-repression' model has been proposed for the mechanism by which phosphorylation activates c-Jun. Thus, c-Jun phosphorylation mediates dissociation of an inhibitory complex which is associated with HDAC3. Subsequent to this dissociation, c-Jun can go on to activate target gene expression whether or not it is phosphorylated. c-Jun can also be activated by an increase in cellular levels of c-Jun protein thereby titrating out limiting components of the repressor complex (40).

c-Jun can itself be the target of acetylation, at least under specific circumstances. The *MMP1* promoter can be activated by c-Jun, and repressed by the adenoviral E1A protein. p300 binds to E1A and repression of c-Jun induction of *MMP1* expression is dependent upon acetylation of c-Jun at Lys271 (15).

A further report also demonstrates that HDACi TSA and SAHA (suberoylanilide hydroxamic acid) can suppress the expression of *c-jun* and therefore the level of c-Jun protein. This in turn leads to decreased binding of c-Jun to promoters of cognate genes such as *COX2* or *MMP1* and therefore HDACi suppress the phorbol ester-induced expression of these genes (41). Data from the Clark laboratory align with this where TSA represses PMA-induced *MMP1* expression in a number of cell lines (MRC5, HeLa, SW1353) (unpublished).

3.2.2. STATs

A recent paper suggests that Stats undergo acetylation at Lys 685 and that this is essential for their dimerisation and nuclear translocation (12). In this case, the action of HDACi would increase acetylation and therefore potentiate Stat signaling. Whilst there are instances of this in the literature (e.g. the IL-4 induction of the 15-lipoxygenase-1 gene, (42), more recent data appear to show the opposite effect in the majority of cases. Thus HDACi have revealed an essential role for HDACs in the transcription of interferon-responsive genes (43-47) since such inhibitors repress IFN-stimulated gene expression.

3.2.3. NFκB

Several *MMP* genes respond to proinflammtory stimuli, at least in part, via the NFκB pathway (e.g. *MMP1*, *MMP3*, *MMP9*, *MMP13* (48). Acetylation can impact upon this signaling pathway at a number of levels. Firstly,

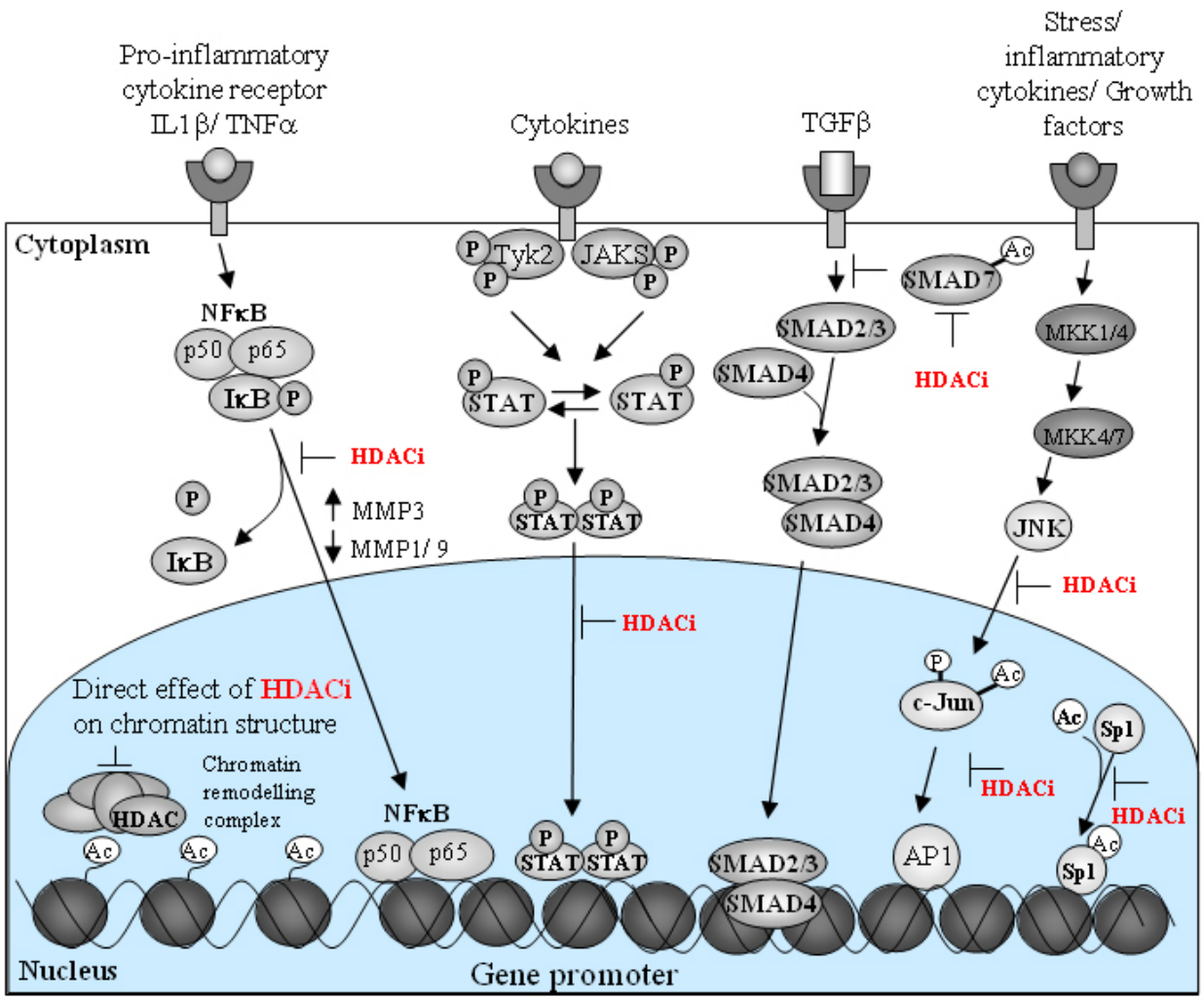

Figure 2. HDAC inhibitors (HDACi) impact upon gene expression via several pathways. Signaling molecules and transcription factors, as well as histones, are modified by acetylation with functional consequences.

NFκB interacts with a number of HATs (including CBP, p300, P/CAF) and HDACs to impact upon gene expression. More specifically, HDACi such as TSA delay postinduction repression of NFκB via prolonged activity of IKK and therefore persistent degradation of IκBα and delayed build up of cytoplasmic IκBα after an inflammatory stimulus. The mechanism of this enhanced IKK activity is unknown (14). Further, both p50 and p65, the most common components of the NFκB dimer can be acetylated at multiple lysine residues. p50 acetylation increases DNA-binding affinity and this correlates with induction of genes such as *COX2* and *iNOS* (14). There are opposing views of the outcome of p65 acetylation. Kiernan et al. (49) show that p65 acetylation reduces DNA-binding affinity and enhances NFκB removal from the nucleus by IκBα and therefore abrogating NFκB action. Chen et al. (50,51) show that p65 acetylation diminishes binding to IκBα, allowing increased nuclear translocation of NFκB and potentiation of signaling. They further show that HDAC3 deacetylates p65 to abrogate signaling and this fits with the ability of HDACi such as TSA to potentiate or prolong NFκB signaling induced by TNFα.

3.2.4. Smad7

Smad 7 can be acetylated on two specific Lys residues by p300 (52). This protects Smad 7 against ubiquitination and proteosomal degradation and therefore abrogates TGFβ signaling. Smad 7 also associates with several HDACs, and at least HDAC1 deacetylates and destabilizes Smad 7 potentially to enhance TGFβ signaling (53).

3.2.5. Other

A number of other transcription factors relevant to *MMP* and *TIMP* gene expression are also subject to regulation at the level of acetylation, including Sp1 and Sp3 and Ets family members (54-58).

4. SUMMARY AND PERSPECTIVE

Changes in acetylation of histones, signalling molecules and transcription factors have profound effects

upon the expression of *MMP* and *TIMP* genes. Modulation of acetylation, using histone deacetylase inhibitors therefore has the potential to modulate these effects with possible therapeutic use in pathologies where MMPs are involved. The current literature, reviewed above, demonstrates that the effects of HDACi are often cell type- and *MMP* gene-specific. The exact HDAC(s) responsible for mediating the impact of a broad-spectrum HDACi on *MMP* gene expression is, in most cases, unknown. If inhibition of HDAC activity is to have therapeutic potential in chronic disease, this information will be crucial to the design of HDAC specific inhibitors. Such targeted compounds may surmount problems of toxicity. Even if such optimism proves unfounded, research in this area will continue to illuminate the complexity of pathways leading to *MMP* gene regulation.

5. ACKNOWLEDGEMENT

IMC would like to acknowledge the support of the Arthritis Research Campaign, the Dunhill Medical Trust and the Norfolk and Norwich Big C Appeal. TES is funded by the Wellcome Trust and the Dunhill Medical Trust. DAY is funded by the JGWP Trust.

6. REFERENCES

1. Egeblad, M. & Z. Werb: New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer*, 2, 161-174 (2002)
2. Chakraborti, S., M. Mandal, S. Das, A. Mandal & T. Chakraborti: Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem*, 253, 269-285 (2003)
3. Wolffe, A. P. & D. Guschin: Review: chromatin structural features and targets that regulate transcription. *J Struct Biol*, 129, 102-122 (2000)
4. Narlikar, G. J., H. Y. Fan & R. E. Kingston: Cooperation between complexes that regulate chromatin structure and transcription. *Cell*, 108, 475-487 (2002)
5. Sudarsanam, P. & F. Winston: The Swi/Snf family nucleosome-remodeling complexes and transcriptional control. *Trends Genet*, 16, 345-351 (2000)
6. Berger, S. L.: Histone modifications in transcriptional regulation. *Curr Opin Genet Dev*, 12, 142-148 (2002)
7. Clayton, A. L., S. Rose, M. J. Barratt & L. C. Mahadevan: Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation. *EMBO J*, 19, 3714-3726 (2000)
8. Kouzarides, T.: Acetylation: a regulatory modification to rival phosphorylation? *EMBO J*, 19, 1176-1179 (2000)
9. de Ruijter, A. J., A. H. van Gennip, H. N. Caron, S. Kemp & A. B. van Kuilenburg: Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J*, 370, 737-749 (2003)
10. Gao, L., M. A. Cueto, F. Asselbergs & P. Atadja: Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. *J Biol Chem*, 277, 25748-25755 (2002)
11. Ng, H. H. & A. Bird: Histone deacetylases: silencers for hire. *Trends Biochem Sci*, 25, 121-126 (2000)
12. Yuan, Z. L., Y. J. Guan, D. Chatterjee & Y. E. Chin: Stat3 dimerization regulated by reversible acetylation of a single lysine residue. *Science*, 307, 269-273 (2005)
13. Yang, X. J. & S. Gregoire: Class II histone deacetylases: from sequence to function, regulation, and clinical implication. *Mol Cell Biol*, 25, 2873-2884 (2005)
14. Quivy, V. & C. Van Lint: Regulation at multiple levels of NF-kappaB-mediated transactivation by protein acetylation. *Biochem Pharmacol*, 68, 1221-1229 (2004)
15. Vries, R. G., M. Prudenziati, C. Zwartjes, M. Verlaan, E. Kalkhoven & A. Zantema: A specific lysine in c-Jun is required for transcriptional repression by E1A and is acetylated by p300. *EMBO J*, 20, 6095-6103 (2001)
16. Gu, W. & R. G. Roeder: Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell*, 90, 595-606 (1997)
17. Gregoretti, I. V., Y. M. Lee & H. V. Goodson: Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J Mol Biol*, 338, 17-31 (2004)
18. Yoshida, M., M. Kijima, M. Akita & T. Beppu: Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J Biol Chem*, 265, 17174-17179 (1990)
19. Kruh, J.: Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Mol Cell Biochem*, 42, 65-82 (1982)
20. Bernstein, B. E., J. K. Tong & S. L. Schreiber: Genomewide studies of histone deacetylase function in yeast. *Proc Natl Acad Sci U S A*, 97, 13708-13713 (2000)
21. Mulholland, N. M., E. Soeth & C. L. Smith: Inhibition of MMTV transcription by HDAC inhibitors occurs independent of changes in chromatin remodeling and increased histone acetylation. *Oncogene*, 22, 4807-4818 (2003)
22. Nair, A. R., L. J. Boersma, L. Schiltz, M. A. Chaudhry, R. J. Muschel & A. Chaudry: Paradoxical effects of trichostatin A: inhibition of NF-Y-associated histone acetyltransferase activity, phosphorylation of hGCN5 and downregulation of cyclin A and B1 mRNA. *Cancer Lett*, 166, 55-64 (2001)
23. Pujuguet, P., D. Radisky, D. Levy, C. Lacza & M. J. Bissell: Trichostatin A inhibits beta-casein expression in mammary epithelial cells. *J Cell Biochem*, 83, 660-670 (2001)
24. Saunders, N., A. Dicker, C. Popa, S. Jones & A. Dahler: Histone deacetylase inhibitors as potential anti-skin cancer agents. *Cancer Res*, 59, 399-404 (1999)
25. Peart, M. J., G. K. Smyth, R. K. van Laar, D. D. Bowtell, V. M. Richon, P. A. Marks, A. J. Holloway & R. W. Johnstone: Identification and functional significance of genes regulated by structurally different histone deacetylase inhibitors. *Proc Natl Acad Sci U S A*, 102, 3697-3702 (2005)
26. Weidle, U. H. & A. Grossmann: Inhibition of histone deacetylases: a new strategy to target epigenetic modifications for anticancer treatment. A*nticancer Res*, 20, 1471-1485 (2000)
27. Chambers, A. E., S. Banerjee, T. Chaplin, J. Dunne, S. Debernardi, S. P. Joel & B. D. Young: Histone acetylation-mediated regulation of genes in leukaemic cells. *Eur J Cancer*, 39, 1165-1175 (2003)
28. Johnstone, R. W.: Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discov*, 1, 287-299 (2002)

29. Monneret, C.: Histone deacetylase inhibitors. *Eur J Med Chem*, 40, 1-13 (2005)
30. Gottlicher, M.: Valproic acid: an old drug newly discovered as inhibitor of histone deacetylases. *Ann Hematol*, 83 Suppl 1, S91-92 (2004)
31. Pender, S. L., J. J. Quinn, I. R. Sanderson & T. T. MacDonald: Butyrate upregulates stromelysin-1 production by intestinal mesenchymal cells. *Am J Physiol Gastrointest Liver Physiol*, 279, G918-924 (2000)
32. Ailenberg, M. & M. Silverman: Trichostatin A-histone deacetylase inhibitor with clinical therapeutic potential-is also a selective and potent inhibitor of gelatinase A expression. *Biochem Biophys Res Commun*, 298, 110-115 (2002)
33. Ailenberg, M. & M. Silverman: Differential effects of trichostatin A on gelatinase A expression in 3T3 fibroblasts and HT-1080 fibrosarcoma cells: implications for use of TSA in cancer therapy. *Biochem Biophys Res Commun*, 302, 181-185 (2003)
34. Kaneko, F., H. Saito, Y. Saito, K. Wakabayashi, N. Nakamoto, S. Tada, H. Suzuki, S. Tsunematsu, N. Kumagai & H. Ishii: Down-regulation of matrix-invasive potential of human liver cancer cells by type I interferon and a histone deacetylase inhibitor sodium butyrate. *Int J Oncol*, 24, 837-845 (2004)
35. Thelen, P., S. Schweyer, B. Hemmerlein, W. Wuttke, F. Seseke & R. H. Ringert: Expressional changes after histone deacetylase inhibition by valproic acid in LNCaP human prostate cancer cells. *Int J Oncol*, 24, 25-31 (2004)
36. Martens, J. H., M. Verlaan, E. Kalkhoven & A. Zantema: Cascade of distinct histone modifications during collagenase gene activation. *Mol Cell Biol*, 23, 1808-1816 (2003)
37. Ma, Z., R. C. Shah, M. J. Chang & E. N. Benveniste: Coordination of cell signaling, chromatin remodeling, histone modifications, and regulator recruitment in human matrix metalloproteinase 9 gene transcription. *Mol Cell Biol*, 24, 5496-5509 (2004)
38. Young, D. A., R. L. Lakey, C. J. Pennington, D. Jones, L. Kevorkian, D. R. Edwards, T. E. Cawston & I. M. Clark: Histone deacetylase inhibitors modulate metalloproteinase gene expression in chondrocytes and block cartilage resorption. *Arthritis Res Ther*, 7, R503-512 (2005)
39. Young, D. A., O. Billingham, C. L. Sampieri, D. R. Edwards & I. M. Clark: Differential effects of histone deacetylase inhibitors on phorbol ester- and TGF-beta1 induced murine tissue inhibitor of metalloproteinases-1 gene expression. *FEBS J*, 272, 1912-1926 (2005)
40. Weiss, C., S. Schneider, E. F. Wagner, X. Zhang, E. Seto & D. Bohmann: JNK phosphorylation relieves HDAC3-dependent suppression of the transcriptional activity of c-Jun. *EMBO J*, 22, 3686-3695 (2003)
41. Yamaguchi, K., A. Lantowski, A. J. Dannenberg & K. Subbaramaiah: Histone deacetylase inhibitors suppress the induction of c-Jun and its target genes including COX-2. *J Biol Chem*, 280, 32569-32577 (2005)
42. Shankaranarayanan, P., P. Chaitidis, H. Kuhn & S. Nigam: Acetylation by histone acetyltransferase CREB-binding protein/p300 of STAT6 is required for transcriptional activation of the 15-lipoxygenase-1 gene. *J Biol Chem*, 276, 42753-42760 (2001)
43. Chang, H. M., M. Paulson, M. Holko, C. M. Rice, B. R. Williams, I. Marie & D. E. Levy: Induction of interferon-stimulated gene expression and antiviral responses require protein deacetylase activity. *Proc Natl Acad Sci U S A*, 101, 9578-9583 (2004)
44. Genin, P., P. Morin & A. Civas: Impairment of interferon-induced IRF-7 gene expression due to inhibition of ISGF3 formation by trichostatin A. *J Virol*, 77, 7113-7119 (2003)
45. Rascle, A., J. A. Johnston & B. Amati: Deacetylase activity is required for recruitment of the basal transcription machinery and transactivation by STAT5. *Mol Cell Biol*, 23, 4162-4173 (2003)
46. Nusinzon, I. & C. M. Horvath: Interferon-stimulated transcription and innate antiviral immunity require deacetylase activity and histone deacetylase 1. *Proc Natl Acad Sci U S A*, 100, 14742-14747 (2003)
47. Sakamoto, S., R. Potla & A. C. Larner: Histone deacetylase activity is required to recruit RNA polymerase II to the promoters of selected interferon-stimulated early response genes. *J Biol Chem*, 279, 40362-40367 (2004)
48. Vincenti, M. P. & C. E. Brinckerhoff: Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res*, 4, 157-164 (2002)
49. Kiernan, R., V. Bres, R. W. Ng, M. P. Coudart, S. El Messaoudi, C. Sardet, D. Y. Jin, S. Emiliani & M. Benkirane: Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. *J Biol Chem*, 278, 2758-2766 (2003)
50. Chen, L., W. Fischle, E. Verdin & W. C. Greene: Duration of nuclear NF-kappaB action regulated by reversible acetylation. *Science*, 293, 1653-1657 (2001)
51. Chen, L. F., Y. Mu & W. C. Greene: Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. *EMBO J*, 21, 6539-6548 (2002)
52. Gronroos, E., U. Hellman, C. H. Heldin & J. Ericsson: Control of Smad7 stability by competition between acetylation and ubiquitination. *Mol Cell*, 10, 483-493 (2002)
53. Simonsson, M., C. H. Heldin, J. Ericsson & E. Gronroos: The balance between acetylation and deacetylation controls Smad7 stability. *J Biol Chem*, 280, 21797-21803 (2005)
54. Braun, H., R. Koop, A. Ertmer, S. Nacht & G. Suske: Transcription factor Sp3 is regulated by acetylation. *Nucleic Acids Res*, 29, 4994-5000 (2001)
55. Ammanamanchi, S., J. W. Freeman & M. G. Brattain: Acetylated sp3 is a transcriptional activator. *J Biol Chem*, 278, 35775-35780 (2003)
56. Huang, W., S. Zhao, S. Ammanamanchi, M. Brattain, K. Venkatasubbarao & J. W. Freeman: Trichostatin A induces transforming growth factor beta type II receptor promoter activity and acetylation of Sp1 by recruitment of PCAF/p300 to a Sp1.NF-Y complex. *J Biol Chem*, 280, 10047-10054 (2005)
57. Czuwara-Ladykowska, J., V. I. Sementchenko, D. K. Watson & M. Trojanowska: Ets1 is an effector of the transforming growth factor beta (TGF-beta) signaling pathway and an antagonist of the profibrotic effects of TGF-beta. *J Biol Chem*, 277, 20399-20408 (2002)
58. Yang, S. H. & A. D. Sharrocks: SUMO promotes HDAC-mediated transcriptional repression. *Mol Cell*, 13, 611-617 (2004)

Key Words: Metalloproteinase, MMP, acetylation, HDAC, trichostatin A, AP1, c-Jun, NFκB, Smad, STAT, Review

Send correspondence to: Ian M Clark, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom, Tel: 44-0-1603 592760, Fax: 44-0-1603 592250, E-mail: i.clark@uea.ac.uk

http://www.bioscience.org/current/vol12.htm