

Irish Area Section (Protein Interactions in Biology)

Targeting cancer using KAT inhibitors to mimic lethal knockouts

James A.L. Brown^{*1}, Emer Bourke[†], Leif A. Eriksson[‡] and Michael J. Kerin^{*}

^{*}Discipline of Surgery, College of Medicine, Lambe Institute for Translational Research, National University of Ireland Galway, H91 YR71, Ireland

[†]Discipline of Pathology, College of Medicine, Lambe Institute for Translational Research, National University of Ireland Galway, H91 YR71, Ireland

[‡]Department of Chemistry and Molecular Biology, University of Gothenburg, 405 30 Goteborg, Sweden

Abstract

Two opposing enzyme classes regulate fundamental elements of genome maintenance, gene regulation and metabolism, either through addition of an acetyl moiety by histone acetyltransferases (HATs) or its removal by histone de-acetyltransferases (HDAC), and are exciting targets for drug development. Importantly, dysfunctional acetylation has been implicated in numerous diseases, including cancer. Within the HAT superfamily the MYST family holds particular interest, as its members are directly involved in the DNA damage response and repair pathways and crucially, several members have been shown to be down-regulated in common cancers (such as breast and prostate). In the present study we focus on the development of lysine (K) acetyltransferase inhibitors (KATi) targeting the MYST family member Tip60 (Kat5), an essential protein, designed or discovered through screening libraries. Importantly, Tip60 has been demonstrated to be significantly down-regulated in many cancers which urgently require new treatment options. We highlight current and future efforts employing these KATi as cancer treatments and their ability to synergize and enhance current cancer treatments. We investigate the different methods of KATi production or discovery, their mechanisms and their validation models. Importantly, the utility of KATi is based on a key concept: using KATi to abrogate the activity of an already down-regulated essential protein (effectively creating a lethal knockout) provides another innovative mechanism for targeting cancer cells, while significantly minimizing any off-target effects to normal cells. This approach, combined with the rapidly developing interest in KATi, suggests that KATi have a bright future for providing truly personalized therapies.

Introduction

Modifications to histones (such as methylation, phosphorylation and acetylation) are used to regulate chromatin structure (relaxing or opening chromatin), ultimately regulating transcription. Importantly, histone acetylation is required for many aspects of gene regulation, metabolism and genome organization/maintenance (for review see [1,2]). Significantly, dysfunctional acetylation has been implicated in numerous diseases, including cancer (for review see [3,4]).

Histone acetylation is primarily regulated by two opposing classes of enzymes, histone acetyltransferases [HATs; also called lysine (K) acetyltransferases (KATs)] and histone deacetylases [HDACs; also known as lysine deacetylases (KDACs)] (Figure 1A). In addition, metabolic regulation of histone acetylation is mediated in part through acetyl-CoA cofactors [5,6]. Currently significant worldwide effort is being expended to investigate the use of HDAC inhibitors for the clinical treatment of cancer [7–9]. However, the therapeutic potential of hindering the opposing machinery, KATs, for the treatment of cancer has only recently been recognized [10–15].

Key words: acetyltransferase, anacardic acid, bisubstrate, breast cancer, curcumin, garcinol, HAT, histone, HTATIP, inhibitor, KAT, Kat5, Lys-CoA, lysine, MG-149, NU9056, pentamidine, TH1834, Tip60.

Abbreviations: ATM, ataxia telangiectasia mutated; DSB, double strand break, HAT, histone acetyltransferase; HDAC, histone de-acetyltransferases; KAT, lysine acetyltransferase; KATi, lysine acetyltransferase inhibitor.

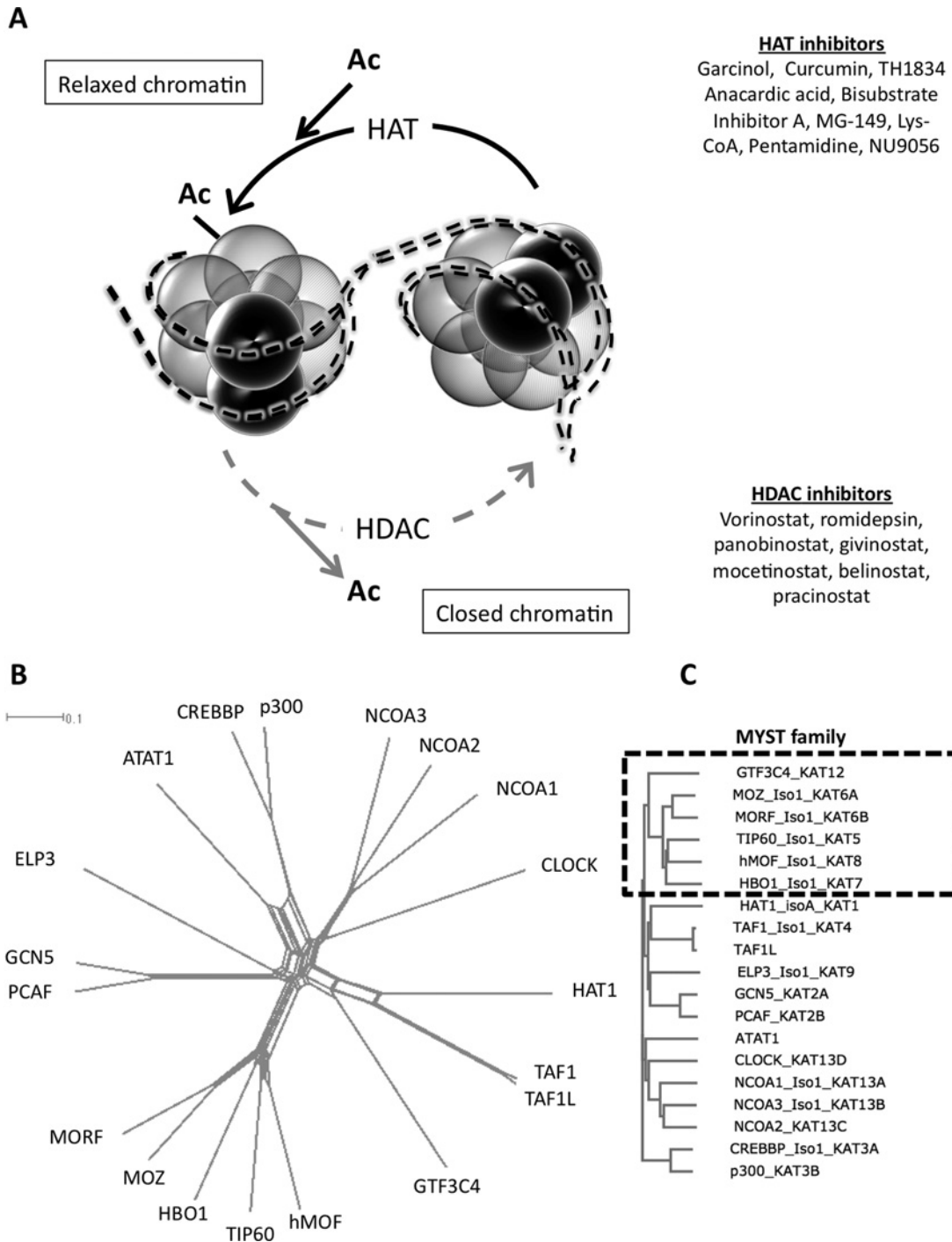
¹ To whom correspondence should be addressed (email james.brown@nuigalway.ie).

KAT family

The KAT family consist of 17 members, as defined by the HUGO gene nomenclature committee. Within this there

Figure 1 | Cycle of histone acetylation/deacetylation and histone acetylation superfamily

(A) Molecular cycle and effects of histone acetylation and de-acetylation. (B) Highlighting the KAT subfamily of HAT's. Analysis by EBI Neighbour-joining clustering method (real phylogram displayed). Generated using SplitsTree4 [64]. (C) Phylogenetic analysis of HAT family members. Labelling: Protein name, isoform (Iso) and gene name.

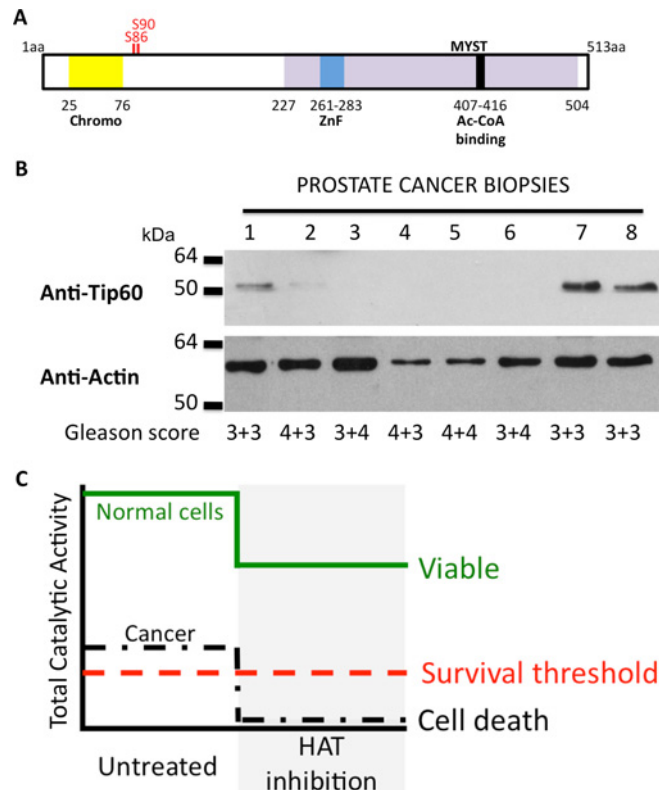


are several distinct families of KATs (based on sequence conservation in the HAT domain), with the largest and most diverse being the MYST family [3,5,10,15–20] (Figures 1B and 1C). The MYST family includes MOZ, YBF2, MOF and

Tip60 (Figure 1C) [21–24]. Membership of the MYST family is defined by the presence of a conserved 3-region histone acetyltransferase domain (containing an acetyl-CoA binding site, a C₂HC zinc finger and a helix-turn-helix DNA-binding

Figure 2 | Tip60 protein structure and expression and cellular consequences of HAT inhibition

(A) Tip60 protein structure. (B) Tip60 expression in prostate cancer biopsies. Thirty micrograms of total protein extracted from prostate cancer biopsies. Antibodies used: anti-Tip60 (K17, Santa Cruz Biotechnology), anti-actin (Abcam). Increasing Gleason scores indicates a worse prognosis in prostate cancer. (C) Model of the mechanism of action allowing HAT inhibitors to preferentially target cancer cells.



motif), responsible for their catalytic histone acetyltransferase activity. Variation between specific members is conferred through the presence of further structural features, such as zinc fingers, PHD fingers and chromodomains. The presence of these additional domains provides an insight into the substrate specificity of the family members. In addition to their well-known role in histone acetylation, the MYST family has a broad substrate range, with new non-histone targets regularly being reported (reviewed in [25,26]). Within the MYST family the importance of Tip60 is highlighted, as a Tip60 knockout is lethal [27]. This essential role for Tip60 is further demonstrated in cancer cells, where down-regulation results in cell death [28,29].

Tip60

The *Kat5* gene encodes Tip60 and isoform 1 (of 4) is a ~60 kDa, 513aa long protein incorporating a histone acetyltransferase domain and a chromodomain (Figure 2A). Tip60 has many diverse substrates, which is reflected in its diverse role in cellular processes. These include the DNA damage response, the cell cycle, apoptosis, signalling and transcriptional regulation (for review see [29–31]).

Importantly, Tip60 auto-acetylation at a key residue in the active site of its MYST domain (K327) regulates, but is not required for, its HAT activity [32,33].

Tip60 and genome stability

A key role of Tip60 is its regulation of the DNA double strand break (DSB) response through acetylation (leading to activation) of the apical kinase ataxia telangiectasia mutated (ATM) and other key DNA damage response and repair proteins (for review see [14,30]). Following a DSB Tip60 is responsible for acetylation of the inactive ATM homodimer, allowing monomerization of active ATM which then initiates the DNA damage response by phosphorylating multiple targets [29,31,34,35]. The importance of the Tip60-dependent activation of ATM is demonstrated following Tip60 knockdown, resulting in an abrogated DSB response and sensitivity to ionizing radiation [36]. Identification of this crucial genome protective role of Tip60 (activating ATM, the DSB response and DNA repair) has led to the proposal that the Tip60 haploinsufficiency observed (in breast and prostate cancer) allows Tip60 to function as an oncogene [27].

Tip60 down-regulation in cancer

Recently it has been demonstrated that several KATs are down-regulated in many different cancers [27,37–39]. Focusing specifically on Tip60, reduced Tip60 transcript expression has been observed in colon, lung, breast and other cancers [10,27,40–43]. Importantly, reduced Tip60 expression was associated with a significantly poorer 5-year disease free survival in primary melanoma patients ($P = 0.016$) and in metastatic melanoma patients ($P = 0.027$) [43]. The same study indicated that Tip60 expression was a significant independent prognostic marker for primary ($P = 0.024$) and metastatic melanomas ($P = 0.035$) [43].

Investigating Tip60 protein levels in cancer, a significant reduction in Tip60 staining (immunohistochemical) has been observed in patient breast and prostate cancer samples [19,27]. Our preliminary data supports this, as we also observed a significant reduction in total Tip60 protein levels in prostate and breast cancer (Figure 2B, Brown et al. unpublished). Interestingly, we observed that Tip60 loss in prostate cancer correlated with an increasing Gleason score (indicating a worse prognosis), a correlation previously demonstrated in gastric cancer [44]. This is further supported by a recent study demonstrating significantly reduced expression (between 130 cancers and 55 controls) ($P = 0.003$) where an increasing reduction in Tip60 mRNA expression correlates with increasing Gleason score [45]. Reduced Tip60 expression has also been observed in breast, melanoma and prostate cancer cells [19,27,46]. In addition, recent unpublished work reported significantly reduced Tip60 levels in lung, pancreatic and breast cancer cell lines (compared with non-tumorigenic controls) [47]. Importantly, although Tip60 is undetectable in some samples previous work demonstrated that loss of Tip60 is lethal, therefore it is likely below the detection threshold of these assays. Together we believe this data suggests and merits testing of the hypothesis: Tip60 loss correlates with increasing disease severity.

Combined, results from multiple cancers indicate that many cancers types have low levels of Tip60 (required for survival) and that lower levels of Tip60 correlate with a worse prognosis. This leads to a novel hypothesis: Eliminating the remaining Tip60 activity in cancer cells (with already reduced protein levels) will cause apoptosis. Importantly, this hypothesis has been confirmed by multiple groups, using a wide range of techniques [1,5,10,15,29]. Consequently, Tip60 is an excellent candidate for targeted drug development of a targeted KAT inhibitor (KATi), which is supported by multiple groups producing targeted inhibitors and testing their efficacy in different cancer types [10,15,18].

KATi

The application of a lysine acetyltransferase inhibitor (KATi) (particularly in Tip60 low cancers) is based on a novel hypothesis: Transiently reducing the activity of a key protein essential for survival (below a crucial minimum threshold) results in death of cancer cells with already reduced levels

of this protein, while allowing normal cells to survive (Figure 2C). Importantly, this hypothesis was recently validated in a breast cancer model [15].

Tip60 targeting KATi can be classed into two broad categories: designed small molecule inhibitors (Bisubstrate Inhibitor A [20], MG-149 [18], TH1834 [15]) or inhibitors from library screens (Lys-CoA [1], garcinol [48], curcumin [1], anacardic acid [3], pentamidine [5], NU9056 [10]) (Table 1). Of these Lys-CoA, anacardic acid, garcinol and curcumin are the best known but least specific, targeting Tip60 in addition to pCAF and CBP/p300 (all at various IC_{50} s) (for review see [11,12,14]).

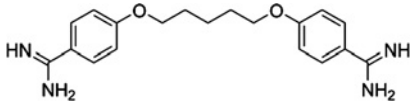
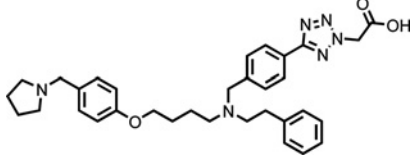
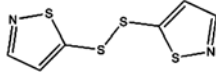
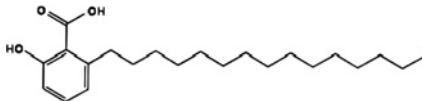
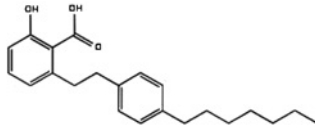
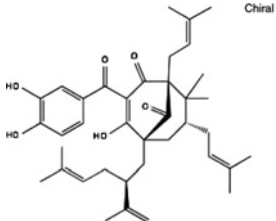
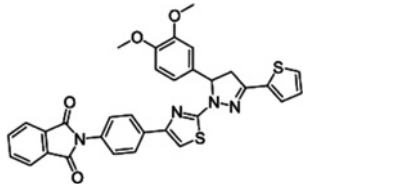
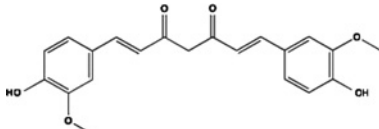
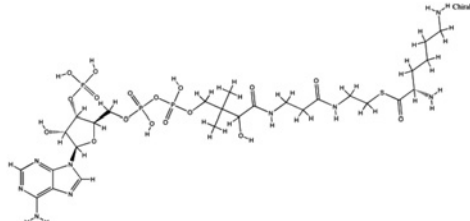
Structural analysis of Tip60 specific KATi

Currently, several inhibitors of Tip60 have been evaluated [3,5,10,15,18–20] (Table 1). Many of these inhibitors are similar in structure to acetyl-CoA, acting as competitive binders.

Several approaches have been used to identify new KATi candidate compounds. One approach is based on using the natural substrate as a core and linking this to various substituents. For example, covalently linking CoA to the lysine residue of a substrate peptide of various chain lengths [21]. This concept of bisubstrates was adopted later by several other groups, producing specific KAT inhibitors. Lys-CoA is obtained by connecting CoA and a single lysine residue via a methylene linker [25]. Lys-CoA is a potent KAT inhibitor as a general bifunctional substrate, with a pronounced selectivity towards p300, but also targeting PCAF and Tip60. Enhanced selectivity can be obtained by tailoring the peptide linker connecting CoA and Lys. To address the MYST family of enzymes, a series of H4 peptide-containing bisubstrate analogues was designed. One of these, H4K16-CoA, was reported as a potent Tip60 inhibitor with an IC_{50} value in the low micromolar range [1]. Unfortunately, however, the compound was also found to display low permeability [1]. Currently, additional targeted design of KATi is underway, requiring testing [49,50].

Another approach is based on screening libraries of isolated natural compounds, which has provided several distinct key KATi, as natural compounds can span a wide range of functionality and complexity. The two main natural Tip60 KATi substances are garcinol and anacardic acid (Table 1). Garcinol is a polyisoprenylated benzophenone isolated from *Garcinia indica* with demonstrated IC_{50} values towards Tip60 in the micromolar range. However, there is a significant lack of selectivity, as the compound displays similar activity towards p300 and PCAF [51]. The molecule has been proposed to exhibit a dual binding mode, based on isothermal calorimetric binding data, with the hydroxy groups of the catechol unit interacting with the acetyl-CoA binding pocket and the isoprenoid units interacting with the substrate binding region [52]. Subsequent modifications to garcinol have been reported, primarily increasing selectivity towards p300 and CBP (low micromolar range) [53].

Table 1 | Tip60 small molecule inhibitors

Compound	Molecular targets	Structure	Reference
Pentamidine	Tip60		[5]
TH1834	Tip60		[15]
NU9056	Tip60		[10]
Anacardic acid	Tip60 PCAF CBP/p300		[3,18,54]
MG-149	Tip60 hMOF		[18]
Garcinol	Tip60		[48]
Bisubstrate Inhibitor A	Tip60		[20]
Curcumin	P300/CBP PCAF Tip60		[1,51]
Lys-CoA	P300/CBP PCAF Tip60		[1]

Anacardic acid is found in the liquid of cashew nut shells and has been identified as a non-selective, non-competitive inhibitor of p300/CBP, PCAF and Tip60 [18]. The inhibitory effect towards its targets is similar under similar experimental conditions, but IC_{50} values vary greatly between reports. The high lipophilicity of anacardic acid is a limiting factor towards its development as a therapeutic agent, with a range of modifications addressing both the salicylic acid moiety and the lipophilic chain proposed in order to enhance selectivity [54]. An example is MG-149 (Table 1), which is one of several 6-alkylsalicylates currently under investigation [18].

Curcumin is another natural substance reported to inhibit Tip60 activity [53]. Curcumin is a major component of *Curcuma longa* rhizome commonly used in Indian and Chinese traditional medicine. It has been reported to exhibit a mode of action involving covalent binding at a site away from the substrate and cofactor binding pocket. Although some selectivity towards different KAT enzymes could be noted, curcumin is a very promiscuous binder inhibiting other epigenetic targets such as lysine (K)-specific demethylase 1A (LSD1), DNA (cytosine-5)-methyltransferase 1 (DNMT1) and KDACs, as well as a wide range of related non-epigenetic proteins [55]. Curcumin is furthermore a known membrane disruptor, and hence some of its activity can most likely be traced to modes of action other than Tip60 binding. A number of analogues to curcumin have been developed aiming at better selectivity and higher water solubility. Although some success is noted, the analogues suffer the same issue of promiscuous binding as the parent compound [56].

Another approach to identify new KATi is *in silico* screening of small molecule databases, using the returned compounds as the basis for further derivatization. A high throughput screening of ~80,000 small molecules led to the production of NU9056. NU9056 specifically inhibits Tip60 activity with an IC_{50} value of 2 μ M. In prostate cancer cells NU9056 treatment induced apoptosis through caspase-3 [10]. Using this method other small molecule inhibitors of Tip60 have also been reported, Lys-CoA and Bisubstrate Inhibitor A [1,20].

An interesting molecule is pentamidine (PNT, Table 1). PNT has been used clinically against parasitic protozoa for over 70 years. Only recently was it reported that DNA and protein synthesis in human tumours was decreased following PNT treatment of whole cell extract, whereby PNT was proposed as an anti-tumour drug [5]. The mode of action is thought to be through inhibition of Tip60 activity, suggested by decreasing histone H2A acetylation and ATM activation, although the exact mechanism requires confirmation [5].

Using PNT as a model compound for Tip60 binding, the modes of interaction of PNT and acetyl-CoA were examined through extensive computational docking as a means of *in silico* drug design [15]. Since several KATs display highly conserved binding pockets, small variations in these could be analysed and used in order to enhance selectivity. This was exploited as the docked structure of PNT was combined with combinatorial chemistry to explore voids in the active site pocket, together with the discovery that the binding pocket

carries differently charged ends interacting with specific amino acids. The resulting compound TH1834, designed entirely from rational drug design, was then synthesized and tested in breast and prostate cancer cells [15]. TH1834 alone induced DNA damage, which importantly was further increased when combined with IR in cancer cells but not in control cells. Mechanistically, it was proposed that the observed increased TH1834-induced γ H2AX foci formation is due to inhibition of chromatin remodelling functions of Tip60 that are required for normal cellular maintenance, and a reduction in Tip60-dependent DNA repair signalling [15].

Targeted rational design, as demonstrated by the validation of TH1834 *in vitro* and in cells, is one *in silico* approach that holds strong promise for the future. This can begin based on known binders, or as a final stage in virtual high-throughput screening campaigns as a means to address selectivity issues found for many families of enzymes. For example, this method of drug discovery is useful for the MYST family enzymes, many ATP binding kinases and the serine protease family, all of which possess active site pockets showing very high structural similarity (within their respective families).

High throughput virtual screening of compounds targeting KAT enzymes resulted in the phthalimide analogue Bisubstrate Inhibitor A (Table 1), based on the acetyl-CoA binding site of the Tip60 yeast homologue Esa1 crystal structure. KAT inhibition efficacy and specificity was assessed using a radiometric *in vitro* assay, showing non-selective inhibitory activity with IC_{50} values in the 100–200 μ M range [20]. It is proposed that selectivity could be enhanced by subjecting the compound to targeted combinatorial chemistry, exploring specific aspects pertaining to the Tip60 active site.

As seen from the examples listed above, although the *in silico* drug design of KAT inhibitors is in its infancy, it demonstrates huge potential. Combining current large databases of compounds (e.g. the ZINC repository, comprising >20 million compounds) and present day screening software with highly parallel supercomputing clusters, screening for potential binders followed by rational design to enhance selectivity offers an attractive initial step prior to experimental synthesis and assays. No doubt, we will in the future see more compounds reported in the literature, where the initial stages of drug design is the result of *in silico* selection and refinement.

The potential of KATi as chemotherapeutics

Currently curcumin, one of the least specific KATi, is the only compound undergoing clinical trials for cancer (for review see [12,57]). Specific Tip60 targeting KATi have been shown to interfere with the DDR, providing additional benefits which can be exploited when Tip60 KATi are combined with other cancer treatments (such as IR and chemotherapeutics) which work through the production of DNA damage [15,28,29,36,58–61]. Indeed, Tip60 dependent acetylation of

E2F1 is required for repair of cisplatin induced DNA damage in human lung carcinoma and osteosarcoma cells [62,63]. Further supporting this, Tip60 inhibition combined with IR induces apoptosis in cervical, breast and prostate cancer cells [5,15].

Conclusions

Further knowledge is required to understand the molecular roles KATs play and the mechanisms that KATs influence, in cancer progression and maintenance. The challenge is translating understanding about these basic mechanisms underpinning cancer into clinically relevant applications, optimally producing a new class of chemotherapeutic drugs that will lead to a major breakthrough for the personalized treatment of cancer. The development of KATi is one such application. Clearly, the use of KATi to target cancer will become a focus for pre-clinical evaluation of cancer treatment. Within this, KATi focusing on Tip60 provide a clear benefit as in general, they specifically target cancer cells over healthy cells, are applicable to a number of common cancers requiring urgent additional treatment options which have been reported to be Tip60 low (i.e. breast and prostate), KATi are more specific and importantly can be combined with current chemotherapeutics for synergistic effect. Furthermore, this is combined with Tip60 as a potential new biomarker, optimally facilitating treatment when paired with a KATi therapeutic.

Funding

JALB and MJK are supported by Breast Cancer Research (BCR), Ireland. EB is supported by NUI Galway Millennium funding. LAE is funded by the Swedish Research Council (VR).

References

- Wu, J., Xie, N., Wu, Z., Zhang, Y. and Zheng, Y.G. (2009) Bisubstrate Inhibitors of the MYST HATs Esa1 and Tip60. *Bioorg. Med. Chem.* **17**, 1381–1386 [CrossRef PubMed](#)
- Marmorstein, R. and Zhou, M.-M. (2014) Writers and readers of histone acetylation: structure, mechanism, and inhibition. *Cold Spring Harb. Perspect. Biol.* **6**, a018762 [CrossRef PubMed](#)
- Sun, Y., Jiang, X., Chen, S. and Price, B.D. (2006) Inhibition of histone acetyltransferase activity by anacardic acid sensitizes tumor cells to ionizing radiation. *FEBS Lett.* **580**, 4353–4356 [CrossRef PubMed](#)
- Farria, A., Li, W. and Dent, S.Y.R. (2015) KATs in cancer: functions and therapies. *Oncogene* **34**, 4901–132015 [CrossRef PubMed](#)
- Kobayashi, J., Kato, A., Ota, Y., Ohba, R. and Komatsu, K. (2010) Bisbenzamidine derivative, pentamidine represses DNA damage response through inhibition of histone H2A acetylation. *Mol. Cancer* **9**, 34 [CrossRef PubMed](#)
- Montgomery, D.C., Sorum, A.W., Guasch, L., Nicklaus, M.C. and Meier, J.L. (2015) Metabolic regulation of histone acetyltransferases by endogenous acyl-CoA cofactors. *Chem. Biol.* **22**, 1030–1039 [CrossRef PubMed](#)
- Johnstone, R.W. (2002) Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat. Rev. Drug Discov.* **1**, 287–299 [CrossRef PubMed](#)
- Martinet, N. and Bertrand, P. (2011) Interpreting clinical assays for histone deacetylase inhibitors. *Cancer Manag. Res.* **3**, 117–141 [PubMed](#)
- Wagner, J.M., Hackanson, B., Lübbert, M. and Jung, M. (2010) Histone deacetylase (HDAC) inhibitors in recent clinical trials for cancer therapy. *Clin. Epigenetics* **1**, 117–136 [CrossRef PubMed](#)
- Coffey, K., Blackburn, T.J., Cook, S., Golding, B.T., Griffin, R.J., Hardcastle, I.R., Hewitt, L., Huberman, K., McNeill, H.V., Newell, D.R. et al. (2012) Characterisation of a Tip60 specific inhibitor, NU9056, in prostate cancer. *PLoS One* **7**, e45539 [CrossRef PubMed](#)
- Simon, R.P., Robaa, D., Alhalabi, Z., Sippl, W. and Jung, M. (2016) KATching-up on small molecule modulators of lysine acetyltransferases. *J. Med. Chem.* **59**, 1249–1270 [CrossRef PubMed](#)
- Manzo, F., Tambaro, F.P., Mai, A. and Altucci, L. (2009) Histone acetyltransferase inhibitors and preclinical studies. *Expert Opin. Ther. Pat.* **19**, 761–774 [CrossRef PubMed](#)
- Dekker, F.J. and Haisma, H.J. (2009) Histone acetyl transferases as emerging drug targets. *Drug Discov. Today* **14**, 942–948 [CrossRef PubMed](#)
- Judes, G., Rifaï, K., Ngollo, M., Daures, M., Bignon, Y.-J., Penault-Llorca, F. and Bernard-Gallon, D. (2015) A bivalent role of TIP60 histone acetyl transferase in human cancer. *Epigenomics* **7**, 1351–1363 [CrossRef PubMed](#)
- Gao, C., Bourke, E., Scobie, M., Famme, M.A., Koolmeister, T., Helleday, T., Eriksson, L.A., Lowndes, N.F. and Brown, J.A.L. (2014) Rational design and validation of a Tip60 histone acetyltransferase inhibitor. *Sci. Rep.* **4**, 1–10
- Selvi, B.R., Chatterjee, S., Modak, R., Eswaramoorthy, M. and Kundu, T.K. (2013) Histone acetylation as a therapeutic target. *Subcell. Biochem.* **61**, 567–596 [CrossRef PubMed](#)
- Marmorstein, R. and Trievel, R.C. (2009) Histone modifying enzymes: structures, mechanisms, and specificities. *Biochim. Biophys. Acta* **1789**, 58–68 [CrossRef PubMed](#)
- Ghizzoni, M., Wu, J., Gao, T., Haisma, H.J., Dekker, F.J. and Zheng, Y.G. (2012) 6-Alkylsalicylates are selective Tip60 inhibitors and target the acetyl-CoA binding site. *Eur. J. Med. Chem.* **47**, 337–344 [CrossRef PubMed](#)
- Halkidou, K., Gnanapragasam, V.J., Mehta, P.B., Logan, I.R., Brady, M.E., Cook, S., Leung, H.Y., Neal, D.E. and Robson, C.N. (2003) Expression of Tip60, an androgen receptor coactivator, and its role in prostate cancer development. *Oncogene* **22**, 2466–2477 [CrossRef PubMed](#)
- Wu, J., Wang, J., Li, M., Yang, Y., Wang, B. and Zheng, Y.G. (2011) Small molecule inhibitors of histone acetyltransferase Tip60. *Bioorg. Chem.* **39**, 53–58 [CrossRef PubMed](#)
- Cole, P.A. (2008) Chemical probes for histone-modifying enzymes. *Nat. Chem. Biol.* **4**, 590–597 [CrossRef PubMed](#)
- Kaidi, A. and Jackson, S.P. (2013) KAT5 tyrosine phosphorylation couples chromatin sensing to ATM signalling. *Nature* **498**, 70–74 [CrossRef PubMed](#)
- Yang, X.-J. (2004) The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res.* **32**, 959–976 [CrossRef PubMed](#)
- Roth, S.Y., Denu, J.M. and Allis, C.D. (2001) Histone acetyltransferases. *Ann. Rev. Biochem.* **70**, 81–120 [CrossRef](#)
- Lau, O.D., Kundu, T.K., Soccio, R.E., Ait-Si-Ali, S., Khalil, E.M., Vassilev, A., Wolffe, A.P., Nakatani, Y., Roeder, R.G. and Cole, P.A. (2000) HATs off: selective synthetic inhibitors of the histone acetyltransferases p300 and PCAF. *Mol. Cell* **5**, 589–595 [CrossRef PubMed](#)
- Sapountzi, V. and Cote, J. (2011) MYST-family histone acetyltransferases: beyond chromatin. *Cell. Mol. Life Sci.* **68**, 1147–1156 [CrossRef PubMed](#)
- Gorini, C., Squatrito, M., Luise, C., Syed, N., Perna, D., Wark, L., Martinato, F., Sardella, D., Verrecchia, A., Bennett, S. et al. (2007) Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. *Nature* **448**, 1063–1067 [CrossRef PubMed](#)
- Hu, Y., Fisher, J.B., Koprowski, S., McAllister, D., Kim, M.-S. and Lough, J. (2009) Homozygous disruption of the Tip60 gene causes early embryonic lethality. *Dev. Dyn.* **238**, 2912–2921 [CrossRef PubMed](#)
- Sun, Y., Jiang, X., Chen, S., Fernandes, N. and Price, B.D. (2005) A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13182–13187 [CrossRef PubMed](#)
- Sapountzi, V., Logan, I.R. and Robson, C.N. (2006) Cellular functions of TIP60. *Int. J. Biochem. Cell Biol.* **38**, 1496–1509
- Sun, Y., Jiang, X. and Price, B.D. (2010) Tip60: connecting chromatin to DNA damage signaling. *Cell Cycle* **9**, 930–936 [CrossRef PubMed](#)

- 32 Yuan, H., Rossetto, D., Mellert, H., Dang, W., Srinivasan, M., Johnson, J., Hodawadekar, S., Ding, E.C., Speicher, K., Abshiru, N. et al. (2012) MYST protein acetyltransferase activity requires active site lysine autoacetylation. *EMBO J.* **31**, 58–70
- 33 Yang, C., Wu, J. and Zheng, Y.G. (2012) Function of the active site lysine autoacetylation in tip60 catalysis. *PLoS One* **7**, e32886 [CrossRef PubMed](#)
- 34 Sun, Y., Xu, Y., Roy, K. and Price, B.D. (2007) DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. *Mol. Cell. Biol.* **27**, 8502–8509 [CrossRef PubMed](#)
- 35 Bennetzen, M.V., Larsen, D.H., Dinant, C., Watanabe, S., Bartek, J., Lukas, J. and Andersen, J.S. (2013) Acetylation dynamics of human nuclear proteins during the ionizing radiation-induced DNA damage response. *Cell Cycle* **12**, 1688–1695 [CrossRef PubMed](#)
- 36 Murr, R., Loizou, J.I., Yang, Y.-G., Cuenin, C., Li, H., Z.-Wang, Q. and Herceg, Z. (2006) Histone acetylation by Trp-1p60 modulates loading of repair proteins and repair of DNA double-strand breaks. *Nat. Cell Biol.* **8**, 91–99 [CrossRef PubMed](#)
- 37 Sakuraba, K., Yasuda, T., Sakata, M., Y.-Kitamura, H., Shirahata, A., Goto, T., Mizukami, H., Saito, M., Ishibashi, K., Kigawa, G. et al. (2009) Down-regulation of Tip60 gene as a potential marker for the malignancy of colorectal cancer. *Anticancer Res.* **29**, 3953–3955 [PubMed](#)
- 38 Cai, M., Hu, Z., Liu, J., Gao, J., Tan, M., Zhang, D., Zhu, L., Liu, S., Hou, R. and Lin, B. (2015) Expression of hMOF in different ovarian tissues and its effects on ovarian cancer prognosis. *Oncol. Rep.* **33**, 685–692 [PubMed](#)
- 39 Sauer, T., Arteaga, M.F., Isken, F., Rohde, C., Hebestreit, K., Mikesch, J.H., Stelljes, M., Cui, C., Zhou, F., Gollner, S. et al. (2015) MYST2 acetyltransferase expression and histone H4 lysine acetylation are suppressed in AML. *Exp. Hematol.* **43**, 794–802 [CrossRef PubMed](#)
- 40 Matterna, L., Escaffit, F., Pillaire, M.-J., Selves, J., Tyteca, S., Hoffmann, J.-S., Gourraud, P.-A., Chevillard-Briet, M., Cazaux, C. and Trouche, D. (2009) The p400/Tip60 ratio is critical for colorectal cancer cell proliferation through DNA damage response pathways. *Oncogene* **28**, 1506–1517 [CrossRef PubMed](#)
- 41 Leonart, M.E., Vidal, F., Gallardo, D., Diaz-Fuertes, M., Rojo, F., Cuatrecasas, M., López-Vicente, L., Kondoh, H., Blanco, C., Carnero, A. and Ramón y Cajal, S. (2006) New p53 related genes in human tumors: significant downregulation in colon and lung carcinomas. *Oncol. Rep.* **16**, 603–608 [PubMed](#)
- 42 Pandey, A.K., Zhang, Y., Zhang, S., Li, Y., Tucker-Kellogg, G., Yang, H. and Jha, S. (2015) TIP60-miR-22 axis as a prognostic marker of breast cancer progression. *Oncotarget* **6**, 41290–41306 [PubMed](#)
- 43 Chen, G., Cheng, Y., Tang, Y., Martinka, M. and Li, G. (2012) Role of Tip60 in human melanoma cell migration, metastasis, and patient survival. *J. Invest. Dermatol.* **132**, 2632–2641 [CrossRef PubMed](#)
- 44 Sakuraba, K., Yokomizo, K., Shirahata, A., Goto, T., Saito, M., Ishibashi, K., Kigawa, G., Nemoto, H. and Hibi, K. (2011) TIP60 as a potential marker for the malignancy of gastric cancer. *Anticancer Res.* **31**, 77–79 [PubMed](#)
- 45 Taylor, B.S., Schultz, N., Hieronymus, H., Gopalan, A., Xiao, Y., Carver, B.S., Arora, V.K., Kaushik, P., Cerami, E., Reva, B. et al. (2010) Integrative genomic profiling of human prostate cancer. *Cancer Cell* **18**, 11–22 [CrossRef PubMed](#)
- 46 Bhoumik, A., Singha, N., O'Connell, M.J. and Ronai, Z.A. (2008) Regulation of Tip60 by ATF2 modulates ATM activation. *J. Biol. Chem.* **283**, 17605–17614 [CrossRef PubMed](#)
- 47 Ravichandran, P. and Ginsburg, D. (2015) Tip60 overexpression exacerbates chemotherapeutic drug treatment in breast, pancreatic, and lung cancer cell lines. *FASEB J.* **29**, s725.21
- 48 Balasubramanyam, K., Altaf, M., Varier, R.A., Swaminathan, V., Ravindran, A., Sadhale, P.P. and Kundu, T.K. (2004) Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression. *J. Biol. Chem.* **279**, 33716–33726 [CrossRef PubMed](#)
- 49 Andreoli, F. and Del Rio, A. (2015) Computer-aided molecular design of compounds targeting histone modifying enzymes. *Comput. Struct. Biotechnol. J.* **13**, 358–365 [CrossRef PubMed](#)
- 50 Yang, C., Ngo, L. and Zheng, Y.G. (2014) Rational design of substrate-based multivalent inhibitors of the histone acetyltransferase Tip60. *Chem. Med. Chem.* **9**, 537–541
- 51 Balasubramanyam, K., Varier, R.A., Altaf, M., Swaminathan, V., Siddappa, N.B., Ranga, U. and Kundu, T.K. (2004) Curcumin a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. *J. Biol. Chem.* **279**, 51163–51171 [CrossRef PubMed](#)
- 52 Arif, M., Pradhan, S.K., Thanuja, G.R., Vedamurthy, B.M., Agrawal, S., Dasgupta, D. and Kundu, T.K. (2009) Mechanism of p300 specific histone acetyltransferase inhibition by small molecules. *J. Med. Chem.* **52**, 267–277 [CrossRef PubMed](#)
- 53 Collins, H.M., Abdelghany, M.K., Messmer, M., Yue, B., Deeves, S.E., Kindle, K.B., Mantelingu, K., Aslam, A., Winkler, G.S., Kundu, T.K. and Heery, D.M. (2013) Differential effects of garcinol and curcumin on histone and p53 modifications in tumour cells. *BMC Cancer* **13**, 37 [CrossRef PubMed](#)
- 54 Souto, J.A., Conte, M., Alvarez, R., Nebbioso, A., Carafa, V., Altucci, L. and de Lera, A.R. (2008) Synthesis of benzamides related to anacardic acid and their histone acetyltransferase (HAT) inhibitory activities. *Chem. Med. Chem.* **3**, 1435–1442 [CrossRef](#)
- 55 Costi, R., Di Santo, R., Artico, M., Miele, G., Valentini, P., Novellino, E. and Cereseto, A. (2007) Cinnamoyl compounds as simple molecules that inhibit p300 histone acetyltransferase. *J. Med. Chem.* **50**, 1973–1977 [CrossRef PubMed](#)
- 56 Vyas, A., Dandawate, P. and Padhye, S. (2013) Perspectives on new synthetic curcumin analogs and their potential anticancer properties. *Curr. Pharm. Des.* **19**, 2047–2069 [PubMed](#)
- 57 Gupta, S.C., Patchva, S. and Aggarwal, B.B. (2013) Therapeutic roles of curcumin: lessons learned from clinical trials. *AAPS J.* **15**, 195–218 [CrossRef PubMed](#)
- 58 Bird, A.W., Yu, D.Y., Pray-Grant, M.G., Qiu, Q., Harmon, K.E., Megee, P.C., Grant, P.A., Smith, M.M. and Christman, M.F. (2002) Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature* **419**, 411–415 [CrossRef PubMed](#)
- 59 Chailleux, C., Tyteca, S., Papin, C., Boudsocq, F., Puget, N., Courilleau, C., Grigoriev, M., Canitrot, Y. and Trouche, D. (2010) Physical interaction between the histone acetyltransferase Tip60 and the DNA double-strand breaks sensor MRN complex. *Biochem. J.* **426**, 365–371 [CrossRef PubMed](#)
- 60 Sun, Y., Jiang, X., Xu, Y., Ayrapetov, M.K., Moreau, L.A., Whetstone, J.R. and Price, B.D. (2009) Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. *Nat. Cell Biol.* **11**, 1376–1382 [CrossRef PubMed](#)
- 61 Bandyopadhyay, K., Banères, J.-L., Martin, A., Blonski, C., Parello, J. and Gjerset, R.A. (2009) Spermidinyl-CoA-based HAT inhibitors block DNA repair and provide cancer-specific chemo- and radiosensitization. *Cell Cycle* **8**, 2779–2779
- 62 Van Den Broeck, A., Nissou, D., Brambilla, E., Eymin, B. and Gazzeri, S. (2012) Activation of a Tip60/E2F1/ERCC1 network in human lung adenocarcinoma cells exposed to cisplatin. *Carcinogenesis* **33**, 320–325 [CrossRef PubMed](#)
- 63 Miyamoto, N., Izumi, H., Noguchi, T., Nakajima, Y., Ohmiya, Y., Shiota, M., Kidani, A., Tawara, A. and Kohno, K. (2008) Tip60 is regulated by circadian transcription factor clock and is involved in cisplatin resistance. *J. Biol. Chem.* **283**, 18218–18226
- 64 Huson, D.H. and Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* **23**, 254–267 [CrossRef PubMed](#)

Received 30 March 2016
doi:10.1042/BST20160081