The second coming of epigenetic drugs

A more strategic and broader research framework could boost the development of new drugs to modify epigenetic factors and gene expression

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Like stem cell research, epigenetics has been promising radical new therapies, aimed at modifying the expression of target genes for a wide range of diseases. New drugs have been slow to materialize, however, largely because epigenetic mechanisms and their role in gene expression are more complex than originally thought. Nonetheless, clinically approved drugs that target epigenetic mechanisms have become available, and over the few past years, the field has entered a new era that some are calling the second generation of epigenetic drug discovery. This new era is characterized by a more coherent framework for epigenetic drug discovery that goes beyond chasing individual targets as they emerge.

The main focus of epigenetic drug discovery efforts has been on cancer, largely because Andrew Feinberg and Ber Vogelstein found altered DNA methylation in cancer in 1983. However, the detailed pathways involved have proved elusive and it has not been easy to separate cause from effect. Meanwhile though, substantial progress has been made understanding and cataloguing the fundamental mechanisms of epigenetic modification of chromatin. These include DNA methylation and histone acetylation, both of which play a key role in gene expression and are implicated in cancer and other conditions.

DNA methylation is one of the most common mechanisms for preventing gene expression. It involves the addition of a methyl group to the fifth carbon atom of the nucleotide cytosine to create 5-methylcytosine (5mC). The methyl group of 5mC can interfere with and block the transcription of genes whose sequences are

encoded nearby on the DNA double helix. In cancer, DNA methylation is almost always highly deregulated, and a notable aspect is that tumours display distinct methylation profiles correlated with their sites in the body [1], even though the clinical significance of this observation is not often well understood.

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Histones are also intimately involved in epigenetic modification, as the DNA in the chromatin is tightly coiled around these structures to organize the genome. DNA has to be first uncoiled from histones in order to be expressed and this is accomplished with the assistance of histone acetyl transferases (HATs). These enzymes add an acetyl group to the lysine residues of core histones, making the surrounding chromatin less compact and more accessible to the transcriptional machinery. Conversely, histone deacetylases (HDACs) remove the acetyl groups from the lysine residues, making the chromatin more condensed, with the effect of silencing DNA transcription. This reversible modification of core histones is the major epigenetic mechanism for remodelling higher-order chromatin structure and, consequently, controlling gene expression. This insight has led to the development of HDAC

inhibitors (HDIs): small molecules that block histone deacetylation, resulting in hyperacetylation of histones in an attempt to prevent gene silencing and alter gene expression.

Histone acetylation also regulates gene expression indirectly through the action of so-called bromodomain and extra-terminal (BET) proteins that recognize and bind to acetylated lysine residues, as found in the N-terminal tails of histones, and subsequently activate transcription factors. Bromodomains have a higher affinity for chromatin regions where multiple acetylation sites exist in proximity and, in this way, bind to target genes. This finding generated excitement initially among leukaemia researchers because molecules that inhibit binding of BET proteins to acetylated histones and thus prevent activation of BET target genes had been shown to attenuate cell growth and prolong survival in several hematologic cancer models [2]. BET inhibitors have also been shown to reduce the expression of the critical oncogene MYC in prostate cancer cell lines from human patients, which inhibits cell growth and reduces tumour volume burden *in vivo* [3]. As a result, BET inhibitors have become a primary focus for cancer research over the past few years.

Apart from modifying the chromatin structure, transcription can also be altered through RNA interference, which has emerged as another important epigenetic mechanism with great therapeutic potential. It operates by silencing genes in the cytoplasm after transcription, or by interfering directly with the transcriptional machinery in the nucleus.

To date, though, chromatin regulation has been the major focus of epigenetic drug development, following

mounting evidence that it is often a cause and not just a consequence of tumorigenesis. “Cancer has been a focus of epigenetic research because of supporting data from TCGA [The Cancer Genome Atlas] and other sources that aberrant chromatin regulation is a driver in some tumours”, commented Stephen Frye, Director of the Centre for Integrative Chemical Biology and Drug Discovery at UNC-Chapel Hill in the USA. TCGA is a partnership between the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) to accelerate our understanding of the molecular basis of cancer.

Frye also stressed the importance of patience and focusing on increasing understanding of epigenetic mechanisms to build a framework for future drug discovery, rather than rushing to develop drugs around existing targets. “As is common, the herd is following along the trodden path”, he said, referring particularly to HDACs and bromodomain antagonists. He did not doubt the potential of these and other epigenetic drugs, but questioned the wisdom of too great an obsession with them at this stage. “Although BRD4 [one member of the bromodomain family] is an exciting target, it may not be the best use of biomedical resources—public and

private—for everyone to be working in this area”, he said. “I’d like to maintain a basic science focus in this area and create a framework for drug discovery in this class. I’m less interested at the moment in rushing in trying to cherry pick the “best” targets and drug them”.

This view is shared by Aled Edwards, Professor at the University of Toronto, Canada, and CEO of the Structural Genomics Consortium (SGC), a public/private partnership involving 200 researchers and spearheaded by the Universities of Oxford, UK, and Toronto, with sponsorship from leading pharmaceutical companies including GSK, Pfizer and Novartis. Edwards has campaigned for more than 10 years for an open access approach to drug discovery work, trying to persuade industry and academia to work together. He emphasized that all parties will benefit from sharing the fundamental research from which drug discoveries emanate.

“It’s not that patenting is bad”, Edwards said. “It’s that open access allows for more innovative drug targets to come to the fore, which provides greater opportunity for drug discoverers, who can then patent to their hearts’ delight. Epigenetic drug discovery is an incredibly high-risk area, and no one knows if it is going to be a cornucopia of

new medicines. And the field is too vast for anyone to own it. Best sort out the “is this area for real” question as a group and then after we know, to invest resources in drug development”.

On the other hand, the success of early epigenetic drugs does provide evidence that it is worth investing in epigenetic therapies. Such evidence is already stronger than the handful of specifically approved epigenetic drugs would suggest, as various existing drugs turn out to have significant epigenetic effects that may account in large part for their efficacy. A recent study found that 1% of all drugs that have been approved by the US FDA show significant epigenetic activity and silence promoters in colon cancer cells [4]. Although this is not a sizable proportion, it equates to about 15 drugs that are already available and that have substantial therapeutic potential.

Among the few drugs that have passed clinical trials specifically for their epigenetic action are Vorinostat from Merck and Romidepsin from Celgene, both for treating cutaneous T-cell lymphoma. Two other HDAC inhibitors, Panobinostat from Novartis and CI-994 from Pfizer, are currently in clinical phase III trials for the treatment of

lymphomas and non-small-cell lung cancer (NSCLC), respectively.

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Vorinostat highlights how relatively broad-spectrum gene silencing can also be effective against seemingly unrelated conditions, such as HIV/AIDS in this case. The evidence has been sufficiently compelling to launch phase 1 and 2 clinical trials sponsored by the University of North Carolina, USA, which is currently recruiting patients. This trial will compare HIV RNA expression and infection within resting (CD4)⁺ cells in HIV-infected patients on stable anti-retroviral therapy before and after a single exposure to Vorinostat, after exposure to short intervals of the drug and after repeated short interval doses over several weeks.

There is also a prominent HDAC inhibitor that was originally approved for a different mode of action as an antiepileptic drug and therapy for bipolar disorders by modulating neurotransmission and intracellular pathways in the brain. But recently, it has been found to inhibit HDAC activity and, as a result, to confer broader protection against various neurodegenerative diseases, including Alzheimer's [5].

However, the versatility of epigenetic actions may come at a price, as side effects can be significant. Such side effects might result from pleiotropy, occurring when one gene has a role in multiple pathways and influences different unrelated phenotypic traits. While silencing a “cancer-causing” gene might stop replication of a tumour cell, for example, it could also cause nausea or worse. However, given the side effects of current cancer treatments, such considerations must be taken in context, and the answer may be to target the drugs to specific chromatin-modifying proteins that are uniquely implicated in a disease.

One such specific drug is showing promise in early trials for the treatment of

an acute form of leukaemia called MLL (mixed lineage leukaemia). The disease occurs when histone methylation targets the wrong genes, which results in abnormal transcriptional activity. A key player here is a methyltransferase found in all eukaryotes called DOT1L, which regulates gene expression through histone methylation. “A universal feature of MLL-rearranged leukaemia is a chromosomal translocation that results in the formation of a fusion protein between the MLL protein and a variety of partner proteins that share the ability to bind DOT1L, recruit this HMT (histone methyltransferase) to aberrant gene locations and thus effect histone methylation and resultant transcriptional activation of leukemogenic genes”, explained Robert Copeland, Executive Vice President and Chief Scientific Officer at Epizyme, the biopharmaceutical company that has developed a drug called EPZ-5676. “EPZ-5676 is a highly potent and selective inhibitor of DOT1L that binds directly to the enzyme at its S-adenosylmethionine binding pocket, thereby blocking enzymatic activity”.

A key point about such drugs is that they inhibit individual HMTs upon which human cancers are utterly dependent, but which play a negligible role in normal cellular activity. “By targeting the enzyme addition of the cancer—an addiction not shared by normal cells—we can achieve very selective impact on cancer cells with minimal effect on non-cancer cells”, Copeland said. “To date, this expectation has been borne out in the phase 1, dose escalation studies of our first two HMT inhibitors to enter clinical testing: EPZ-5676, an inhibitor of DOT1L, and EPZ-6438, an inhibitor of EZH2 [another HMT involved in some leukaemias]”.

Epigenetic research is not just focused on finding therapeutics though. Given that prevention is better than cure, there has been particular interest in probing how epigenetic changes associated with cancer and other conditions arise in the first place. A key breakthrough came in 2009 with a paper that identified a clear molecular link between inflammation and cancer [6], according to Claude Gérard, an epigenetics researcher at the Catholic University of Louvain in Belgium. “Previous studies had already identified links between inflammation and cancer development”, Gérard said.

“However, to my knowledge, Iliopoulos and co-workers brought to light one of the first molecular pathways linking inflammation to cell transformation”.

More recently, Gérard and colleagues have developed a computer model of the particular molecular pathway that links inflammation to cell transformation. The model indicates that a semi-stable epigenetic switch plays a central role in the process that leads from inflammation to transformation associated with cancer. “This switch is called irreversible because once the cell switches to a transformed state as a consequence of a sufficiently strong inflammatory signal, this state can be maintained even if the initial inflammatory signal is completely removed”, Gérard said. “However, as observed in the experiments and supported by the mathematical model, the cell can revert back to a non-transformed state if any of the activators of the switch, such as NFκB, Lin-28, miR-21, IL-6 or STAT3, are transiently inhibited”. This suggests that a number of different components of the pathway could be targeted in an attempt to reverse cell transformation.

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One significant finding was that random fluctuations in gene expression that occur within cell populations can spontaneously switch a cell to a transformed state. “Stochastic transitions between different phenotypic states of the cell have been highlighted in different situations”, Gérard noted. He stressed, however, that this does not mean people get cancer just through bad luck as a result of unfortunate random variations in gene expression. “[T]his epigenetic switch can be observed only if cells have already accumulated sufficient genetic alterations”, Gérard explained. “Once cells have those mutations, our model shows that random fluctuations in gene expression can switch the phenotype of the cell to a transformed state. Furthermore, the model also predicts that an increase in the level of the tumour suppressor Let-7 can reduce the occurrence of spontaneous switches (stochastic transition) to a transformed state of the cell”. The

therapeutic implication might be that at-risk people who have accumulated certain mutations could then be given drugs that target the associated epigenetic switch to avoid spontaneous cellular transformation. At any rate, the model sheds more light on how random variations in gene expression, or even in response to changing environmental or metabolic circumstances, can trigger cancer.

Amedeo Caflisch, Professor for Computational Structural Biology at the University of Zurich in Switzerland commented that computational methods could also be used for specific epigenetic drug discovery, provided the structure of the target proteins is known. “Whenever the crystal structure of the protein target is known, *in silico* screening is more efficient and less expensive for identifying hits than *in vitro* screening”, he said. His own team have been targeting human bromodomains for drug discovery. Caflisch argued that computational methods are more selective because they start from the target directly and then proceed to identify ligands fragment by fragment by working through the crystal structure of the target bromodomain. He claimed that his team had just succeeded in developing a selective inhibitor of a

bromodomain called CREBBP, in work still unpublished.

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Such computational methods will play a big role in the expanding field of epigenetic drug discovery. Yet, on a more strategic level, initiatives such as the SGC provide both a more general framework for drug discovery and a platform for academia and industry to work together. After some initial excitement, the field is now being driven by a more healthy balance of optimism and realism through greater collaboration between pharma and academia. Major drug companies can present this as evidence that they are developing a new generation of more personalized therapies instead of broad-spectrum blockbusters while academics can advance their work faster with better funding and collaboration.

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