Institution: The University of Edinburgh

Unit of Assessment: UoA5: Biological Sciences

Title of case study:

02. Understanding of DNA methylation leads to (a) new tools and reagents brought to market and (b) clinical trials for cancer treatments

1. Summary of the impact

Impact on commerce: A patented technique for separating methylated and non-methylated DNA has been licensed and a kit brought to market, along with other commercial reagent licenses.

Impact on health and welfare: The demonstration that two mechanisms of epigenetic gene regulation, DNA methylation and histone acetylation, are linked, has led to trials of separate drugs known to affect each mechanism as a combined treatment for high-risk patients with myelodysplastic syndromes (MDS).

Beneficiaries: Companies have gained commercial benefit from licensing UoE IP to market products. High-risk MDS patients will benefit from improved treatment.

Significance and Reach: Commercial earnings across 4 companies from international sales in the period estimated at over [text removed for publication], mainly since 2010. Commercial significance includes the first commercially-available technique for separating methylated and non-methylated DNA.

The incidence of MDS is estimated at 3-4 cases diagnosed annually per 100,000 of the population in Europe (an estimated 26,000 individuals) and up to 20,000 new diagnoses per year in the USA. Incidence increases with age – up to 15 new cases annually per 100,000 in individuals aged over 70 years. MDS occurrence is increasing as the age of the population increases, so the significance of new therapies is high.

Attribution: All research was led by Adrian Bird at UoE. Reik (Babraham Institute) contributed to development of one of the licensed antibodies.

2. Underpinning research

Research at UoE led by Adrian Bird discovered the protein MeCP2 (methyl CpG binding protein 2) and in 1997 showed that it can act as a transcriptional repressor [1]. DNA Methylation sites occur predominantly in CpG dinucleotides that are mainly located in CpG islands (CGIs) near gene promoters. CGIs are not usually methylated, unlike the bulk of the genome; however if methylation of the CGIs occurs, transcription of the related gene is almost always silenced.

In 1998 the UoE research showed that MeCP2 acts as a transcriptional repressor by recruiting histone modification factors to its binding site [2]. This finding established for the first time a mechanistic connection between DNA methylation and transcriptional repression by the modification of chromatin. Until this, no one knew how methylation affected chromatin structure and in fact there was no known link between DNA methylation and histone acetylation. The research demonstrated that MeCP2 exists as a complex with histone deacetylases and indicated that it provided a mechanistic bridge between DNA methylation and histone deacetylation. This was an important breakthrough in understanding the mechanisms of epigenetic gene silencing and was the first time that two global mechanisms of gene regulation, DNA methylation and histone deacetylation, were shown to be linked. DNA methylation and histone deacetylation modification are now known as the key molecular processes in epigenetic silencing and their linkage has implications for cancers caused by hyper-methylation and silencing of multiple genes.

In further studies of DNA methylation, the Bird group invented a method for separating methylated and unmethylated CpG dinucleotides. This technique enabled them to show that only half of CGIs occur at the beginning of genes, near the promoter, and the rest occur within or between genes. This was an unexpected result. The research went on to show that genes whose protein products play a vital role during embryonic development are preferentially methylated. This suggested that

DNA methylation in CGIs regulates gene expression during development [3]. A patent was filed on the method for separating methylated and non-methylated DNA used in this research [4].

In the same period, the Bird lab discovered the methyl-CpG binding domain (MBD) proteins [5] and developed a number of MBD antibodies (sheep anti-human MBD1 antibody; sheep anti-mouse MBD2 antibody; rabbit anti-mouse MBD2 antibody) developed as part of studies reported in several papers e.g. [6]. In collaboration with Wolf Reik at the Babraham Institute, Cambridge in 2010 they made a rat monoclonal antibody raised against 5-hydroxymethylcytosine (5-hmC). 5-hmC is a modified form of cytosine recently found in mammals and is believed to play an important role in gene expression, distinct from 5-methylcytosine (5-mC). This antibody was developed specifically to distinguish 5-hmC from 5-mC as conventional methods cannot do so.

Key personnel at UoE: Professor Adrian Bird (1990-present); Xinsheng Nan, PDRA (1991-1998) and (2004-2005); Rob Illingworth, PhD student then PDRA (2003-2010); Helle Jorgensen, PDRA (2000-2004); Brian Hendrich, PDRA (1994-2001); Huck-Hui Ng, PhD student (1996-1999). Others contributing substantially to the research: Carol Laherty & Fred Eisenman, Fred Hutchinson Cancer Research Center, Seattle [2]; P. Ellis and others of the Sanger Centre, Cambridge [sequencing data, 4]; Wolf Reik of the Babraham Institute, Cambridge [rat monoclonal antibody].

3. References to the research

1. Nan, X., Campoy, F.J., and Bird, A. (1997). MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell* **88**, 471-481. *doi*:10.1016/S0092-8674(00)81887-5

   599 Scopus citations on 24/10/2013.

2. Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N., and Bird, A. (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393**, 386-389. doi:10.1038/30764

   1789 Scopus citations on 24/10/2013.

3. Illingworth R, Kerr A, DeSousa D, Jørgensen H, Ellis P, et al. (2008) A novel CpG island set identifies tissue-specific methylation at developmental gene loci. *PLoS Biol* 6(1): e22. doi:10.1371/journal.pbio.0060022

   220 Scopus citations on 24/10/2013.

4. Patent PCT/GB2005/004202

   Inventors: Adrian Bird, Robert Illingworth, Helle Jorgensen.

   Granted in USA (US 08105787 B2) “Applications of nucleic acid fragments” 31st January 2012 and under examination in Europe as (EP1807533 A2) “Applications of isolated nucleic acid fragments comprising CpG islands”.

5. Hendrich, B., & Bird, A. (1998). Identification and characterization of a family of mammalian methyl-CpG binding proteins. Molecular and cellular biology, 18(11), 6538–6547. PubMedID: 9774669

   678 Scopus citations on 24/10/2013.

6. Ng, H. H., Jeppesen, P., & Bird, A. (2000). Active repression of methylated genes by the chromosomal protein MBD1. Molecular and cellular biology, 20(4), 1394–1406. DOI: 10.1128/MCB.20.4.1394-1406.2000

   174 Scopus citations on 24/10/2013.

4. Details of the impact

Impact on commerce: licenses awarded and brought to market with sales of new products

IP developed by UoE during this research programme has led to several commercial products. A patent [4] was filed in 2005 covering the method for separating methylated and non-methylated DNA as described and used in the 2008 Illingworth paper [3]. This was granted in USA in 2012 and

is currently under examination in Europe. Five evaluation licences were negotiated with companies developing reagents in this area, [text removed for publication]). Two royalty-bearing licences were awarded in 2012 to [text removed for publication]. ActiveMotif have now brought a product to market using this technology – the **UnMethylCollector™ Kit**. [a]

This is the first commercially available kit for the specific isolation and enrichment of unmethylated CpG dinucleotides. Previously, researchers looking to identify unmethylated DNA have had to depend on negative data from methyl-specific binding techniques. Use of the UnMethylCollector kit allows positive identification of unmethylated regions and provides DNA suitable for use in many downstream applications, such as real time or endpoint PCR analysis of the methylation status of particular loci, sequencing, or amplification and labelling for microarray analysis. Analysis of the methylation status of a specific genomic DNA locus can be performed on DNA isolated from less than ~10 ng DNA and the specificity is able to enrich for DNA fragments containing only a single unmethylated CpG. Royalties received by UoE as a percentage of commercial sales revenue equate to an estimated commercial value of [text removed for publication] in the period.

UoE also licenced three methyl-CpG binding domain (MBD) antibodies arising from work in the Bird lab [5, 6] to [text removed for publication] in 2004. License agreements for the rat monoclonal antibody raised against 5-hydroxymethylcytosine (5-hmC), which was developed with the Babraham Institute, were signed in 2010 with [text removed for publication]. At this time 5-hmC was newly-discovered and existing methods could not distinguish methylcytosine (5-mC) from 5-hmC. Product developers thus had to develop alternate ways to detect the modified base, and the [text removed for publication] rat antibody was the first monoclonal antibody available for locus-specific 5-hmC analysis [b,c]. Subsequent licenses were signed with [text removed for publication] in 2011. The net commercial sales revenue to the companies over the REF census period from sales of these antibodies estimated from royalty value is approximately [text removed for publication].

Impact on health: Clinical trials for improved cancer treatments

Treatment of patients with drugs that influence epigenetic control of gene expression is at the forefront of new approaches to cancer therapy. Discovering that two global mechanisms of gene regulation, DNA methylation and histone deacetylation, were linked [2] was an important breakthrough in understanding the mechanisms of epigenetic gene silencing. This is particularly so for clinical research and development of new epigenetic therapies, given that altered DNA methylation patterns are an important characteristic of cancer development (cancer cells use hyper-methylation to silence the expression of tumour suppressor genes). The Nan *et al*. paper [2] was one of the fundamental findings that has led to increased understanding of the role of epigenetic modifications in cancer and to new approaches, which are exemplified in the trials described below, to combining therapies that address different epigenetic modifications in order to improve the efficacy of cancer drugs [as set out, for instance, in e.g. Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB, *Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer*. Nat Genet. 1999; 21:103–107; Kelly TK, De Carvalho, DD, and Jones, PA, *Epigenetic Modifications as Therapeutic Targets*. Nat Biotechnol. 2010 28(10): 1069–1078; *Epigenetic therapy: use of agents targeting deacetylation and methylation in cancer management*. Ho, AS, Turcan, S, and Chan, TA, Onco Targets Ther. 2013; 6: 223–232].

The linkage between methylation and histone deacetylation revealed by the UoE research [2,3] has underpinned subsequent findings that the combination of a hypomethylating agent and deacetylase inhibitor is synergistic for gene reactivation *in vitro*. [e.g. Gore, 2009, Leukaemia Research Volume 33, Supplement 2, pp. S2–S6]. This has led to trials of combined agents as cancer therapies.

Azacitidine, known by its trade name Vidaza, functions as a methylation inhibitor by being incorporated into DNA, inhibiting DNA methyltransferase and causing hypo-methylation of DNA. Vidaza is used as a chemotherapy agent to stop the inappropriate gene silencing in cancer cells and to cause re-expression of tumour suppressor genes. Vidaza is used to treat a group of cancers known as myelodysplastic syndromes (MDS) that affect the bone marrow and blood. It can also be used to treat chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML). These diseases are characterised by the hyper-methylation and silencing of multiple genes.

Panobinostat is a non-selective histone deacetylase (HDAC) inhibitor developed for the treatment of various cancers. It has little effect on its own in MDS patients. However, the linkage between methylation and histone acetylation revealed by the 1998 UoE research has led several international groups to undertake trials using Vidaza and Panobinostat in combination to seek an effective treatment to improve outcomes in higher risk patients with MDS, CMML and AML. Four clinical trials are now underway.

Preliminary data from two trials presented at the American Society of Haematology (ASH) 2011 annual meeting showing that Panobinostat and Vidaza in combination is well tolerated and demonstrates clinical activity in previously untreated MDS patients [d]. One Phase Ib/II trial, presented by Dr Peter Tan of the Alfred Hospital, Melbourne, Australia treated MDS and AML patients throughout Germany and Australia who were not fit for standard intensive chemotherapy and found that five days of treatment with Vidaza followed by 30mg Panobinostat was well tolerated [e]. Another trial at phase I presented by Dr Oliver Ottmann of the Goethe University, Frankfurt, Germany treated MDS, AML and CMML patients in Germany, France and multiple centres in the USA. This study found that 30mg Panobinostat used with a seven day Vidaza schedule may be effective and safe [f]. A phase Ib clinical trial (NCT01613976) sponsored by Novartis is underway throughout Japan of the combined use of Panobinostat and Vidaza in 12 MDS, CMML and AML patients [g]. The trial started in August 2012 and the final date for data collection is September 2013. Another Phase Ib/IIb trial (NCT00946647) started in December 2009 and is due to complete data collection in August 2013 [h]. This multi-centre trial is treating 111 patients in forty-five locations including eight locations across the USA, nine in Europe, two in Canada, Thailand, Korea and Sweden.

5. Sources to corroborate the impact

The Tiny URLs provide a link to archived web content, which should be accessed if the original web content is no longer available

[a] UnMethylCollector product information: http://www.activemotif.com/catalog/550/unmethylcollector or http://tinyurl.com/mcxzlbg

[b] 5-hmC m-ab product information: http://www.diagenode.com/en/catalog/dna-methylation-65/antibodies-68/product/5-hmc-monoclonal-antibody-rat-classic-2351 or http://tinyurl.com/mt836ww

[c] 'Epigenie' Reviews on Diagenode's 5-hmC monoclonal antibody: http://epigenie.com/expert-insight-5-hmc-analysis-methods/ or http://tinyurl.com/kcxjgu3 and http://epigenie.com/doubling-down-on-5-hmc-profiling-with-dual-medip-from-diagenode/ or http://tinyurl.com/kc3mkwn

[d]. Report in the newsletter for the myelodysplastic syndromes community 'MDS Beacon': http://www.mdsbeacon.com/news/2012/02/06/panobinostat-and-vidaza-azacitidine-combination-may-be-effective-in-higher-risk-myelodysplastic-syndromes-patients-ash-2011/ or http://tinyurl.com/lwpnhyd

[e] 58th Annual ASH meeting abstracts: https://ash.confex.com/ash/2011/webprogram/Paper38715.html or http://tinyurl.com/leywu6s

[f] 58th Annual ASH meeting abstracts. https://ash.confex.com/ash/2011/webprogram/Paper37915.html or http://tinyurl.com/k8o5hqx

[g] Phase 1b trial: http://clinicaltrials.gov/ct2/show/NCT01613976?recr=Open&cond=%22Myelodysplastic+Syndromes%22&intr=%22panobinostat%22+%22vidaza%22&rank=2 or http://tinyurl.com/l93l86x

[h]. Phase Ib/IIb trial: http://clinicaltrials.gov/ct2/show/study/NCT00946647?recr=Open&cond=%22Myelodysplastic+Syndromes%22&intr=%22panobinostat%22+%22vidaza%22&rank=1&show_locs=Y#locn or http://tinyurl.com/m5wguyq