Evolution of a contagious cancer: epigenetic variation in Devil Facial Tumour Disease

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The emergence of Devil Facial Tumour Disease (DFTD), a highly contagious cancer, is driving Tasmanian devils (Sarcophilus harrisii) to extinction. The cancer is a genetically and chromosomally stable clonal cell line which is transmitted by biting during social interactions. In the present study, we explore the Devil Facial Tumour (DFT) epigenome and the genes involved in DNA methylation homeostasis. We show that tumour cells have similar levels of methylation to peripheral nerves, the tissue from which DFTD originated. We did not observe any strain or region-specific epimutations. However, we revealed a significant increase in hypomethylation in DFT samples over time (p < 0.0001). We propose that loss of methylation is not because of a maintenance deficiency, as an upregulation of DNA methyltransferase 1 gene was observed in tumours compared with nerves (p < 0.005). Instead, we believe that loss of methylation is owing to active demethylation, supported by the temporal increase in MBD2 and MBD4 (p < 0.001). The implications of these changes on disease phenotypes need to be explored. Our work shows that DFTD should not be treated as a static entity, but rather as an evolving parasite with epigenetic plasticity. Understanding the role of epimutations in the evolution of this parasitic cancer will provide unique insights into the role of epigenetic plasticity in cancer evolution and progression in traditional cancers that arise and die with their hosts.

1. Introduction

The recent appearance of a clonally transmissible cancer, Tasmanian Devil Facial Tumour Disease (DFTD) threatens the Tasmanian devil (Sarcophilus harrisii) with extinction within the next 25 years [1–5]. The main characteristics of this progressive disease are malignant tumours around the face, jaw and in the oral cavity, as well as frequent occurrence of metastases (65% of cases) of the primary tumours [6]. Starvation, secondary infection and metastases usually lead to the death of animals within 6 months of the emergence of the first lesions [3,7]. Cytogenetic analyses have revealed that DFTD is caused by a rogue cell line—an allograft [8], which emerged once at least 15 years ago and has been transmitted from animal to animal by cell implantation during biting [9]. Large-scale genetic [10] and immunohistochemistry [11] analyses suggest that DFTD is clonally derived from cells in the neural crest lineage, most probably Schwann cells. Devil Facial Tumour (DFT) cells in different individuals are genetically identical at microsatellite markers and major histocompatibility complex (MHC) genes [12–15]. The recent sequencing of
the Tasmanian devil and DFT genomes [16,17] has also demonstrated very low levels of genetic polymorphism in both devils and DFT cells [16,17]. Although DFTs possess complex chromosomal changes, these chromosomal rearrangements are highly conserved between tumours [8,18]. Four closely related but karyotypically distinct DFT strains have recently been described, suggesting that although this cancer appears to be stable, it is evolving [16–19].

While the genetic and karyotypic changes of DFT cells have been well documented [16–19], the role of epigenetics in DFT evolution has not yet been explored. Epigenetic processes (mitotically and meiotically heritable changes in gene expression that are not caused by changes in the primary DNA sequence) contribute to the development and evolution of human cancer [20,21]. They are driven by DNA methylation and involve a biochemical modification of cytosines via the addition of a methyl group to the five-carbon position of cytosine pyrimidine rings [22–24]. DNA methylation is essential for the regulation of gene expression and genomic stability [25]. Recent reviews have extensively described the dual nature of cancer epigenetics which includes: (i) tumour cells exhibiting loss of global methylation (global hypomethylation), leading to reactivation of oncogenes; and (ii) gains of methylation (region-specific hypermethylation) at promoters and regulatory regions of genes, resulting in the silencing of tumour-suppressor genes [26,27]. DNA methyltransferase (DNMT) enzymes have been shown to be responsible for addition of methyl groups to cytosine at the five-carbon position [28]. In mammalian cells, DNA methylation is catalysed by two classes of DNMTs: DNMT1 is essential for maintaining DNA methylation patterns in proliferating cells, while the other members of the second class of methyltransferases, DNMT3a and DNMT3b are required for de novo methylation during embryonic development [23]. The mechanism behind hypomethylation is less well understood and still controversial [23,29]. Apart from other mechanisms (e.g. lack of DNMT activity, DNA strand break and/or nucleoside excision repair of DNA [30,31]) two enzymes, MBD2 and MBD4 have been shown to cause active DNA demethylation [31–33]. Methyl-CpG binding domain (MBD) proteins are purported to silence genes by recruiting histone deacetylase to transform chromatin to a repressive state [31–33]. MBD4 has also been proposed to function as a DNA T : G mismatch repair enzyme and may have a role in minimizing mutation at methyl-CpG sites [34].

In this study, we explore the DFT epigenome and the genes involved in DNA methylation homeostasis.

2. Material and methods

(a) Samples
Thirty five tumour and 12 tissue samples were obtained from a total of 41 individual Tasmanian devils (detailed list of samples used in the manuscript can be found in the electronic supplementary material, table S1). The karyotype of DFT samples was determined using the procedure described by Pearse et al. [8,19]. Tissue samples came from eight infected individuals and four uninfected individuals and consisted of one liver, two brain, two heart, two kidney and five sciatic nerve samples. Gene expression analyses were carried out on five nerve and two heart, two kidney and five sciatic nerve samples. Gene expression analyses were carried out on five nerve and two heart, two kidney and five sciatic nerve samples. Gene expression analyses were carried out on five nerve and two heart, two kidney and five sciatic nerve samples. Gene expression analyses were carried out on five nerve and two heart, two kidney and five sciatic nerve samples. 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Gene expression analyses were carried out on five nerve and two heart, two kidney and five sciatic nerve samples.
methylated or hemimethylated, but would not if the 5′ cytosine is fully or hemimethylated. Experimental procedures followed the methodology described by Lo et al. [40]. Detailed description of methods and primer sequences can be found in the electronic supplementary material, S3 and table S4. AFLP and methylation-specific amplified fragment length polymorphism (metAFLP) fragments were separated and visualized using an ABI 3130XL Genetic Analyzer, and scored using the program GeneMarker (SoftGenetics, LLC). Peaks were scored independently by two examiners and only unambiguously distinguishable peaks were accepted.

(c) Quantitative real-time PCR
To understand the potential epigenetic changes and the underlying mechanisms in DFTD cells, we measured expression levels of two DNMTs (DNMT1 and DNMT3a) and two DNA-demethylation enzymes, MBD2 and MBD4 using quantitative-PCR (Q-PCR) technique. RNA was extracted from tissue samples using a combination of Trizol (Sigma) and Qiagen RNEasy mini kit (Qiagen). RNA quality and quantity was measured using Agilent 2100 Bioanalyzer (Agilent). Genomic DNA was removed from the RNA samples by using the DNase I AMPD1 kit (Sigma) and cDNA was synthesized with the QuantiTect Reverse Transcription Kit (Qiagen). Gene-specific primers (DNMT1_v2, DNMT3a_v1, MBD2_v2, MBD4_v2) spanning across exon boundaries were designed based on the Devil Genome (ENSEMBL [17]), using the Primer3Plus website (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi; electronic supplementary material, table S4). Two genes, GAPDH (gGAPDH) and GUSB (gGUSB) were used as normalizer genes following the description of Murchison et al. [10] (see the electronic supplementary material, table S4). The Q-PCR reactions were performed on the RotorGene6000 (Qiagen) using the Qiagen 2xQuantifast Sybr Green PCR master mix (Qiagen). Details are provided in the electronic supplementary material.

(d) Statistical analyses
Relative quantification was performed using sample crossing points, and data were analysed with the RoToR Gene 6000 software v. 1.7. (Qiagen), applying the ‘second derivative maximum’ method [41]. The Excel application BEST-KEEPER [42] was used to check the data for statistical significance, normality and reliability, and the normalizer gene GUSB was chosen as a reference based on BEST-KEEPER calculations [42]. The program REST [43] was used to calculate the normalized fold change of target genes compared with the reference gene. Statistical significance (p < 0.05) was determined by a pairwise fixed reallocation randomization test as described by Pfaffl et al. [43]. Hierarchical analyses of molecular variance and minimum spanning networks were computed using ARLEQUIN v. 3.11. to evaluate tumour epigenetic population structure. In other statistical analyses, data were examined for normality before analysis and when normality could not be achieved, non-parametric statistics were employed. Analyses were carried out using JMP v. 5.1 (SAS institute 1998). Population genetic analysis was conducted by using the software ARLEQUIN v. 3.11. [44].

3. Results

(a) Comparisons of amplified fragment length polymorphisms in tumour and devil tissues
Using four primer combinations, a total of 251 AFLP sites were scored in the 35 tumour and the 12 tissue samples. Sixteen variable AFLP sites were observed in the 35 tumour samples, while 89 polymorphic sites were recorded in the 12 tissue samples. The number of polymorphic AFLP sites were significantly higher in tissue compared with tumour samples (χ² = 64.2, p < 0.0001). However, 83 of the 89 variable tissue AFLP sites were observed in the five nerve samples and only eight variable AFLP sites were recorded in the seven other tissue samples. Thus, the five nerve samples showed a significantly higher level of polymorphic AFLP sites than the seven tissue samples (liver, brain, heart and kidney; χ² = 75.5, p < 0.0001). When restricting the comparison of number of variable AFLP sites of the 35 tumour and that of the seven liver, brain, heart and kidney samples, no significant difference was observed (χ² = 2.8, p = 0.09; table 1).

(b) Comparison of methylation in tumour and devil tissues
We observed a significantly higher mean number of combined methylation sites in the 35 tumours compared with the 12 tissue samples (mean: 24.8 and 18.8, respectively; unpaired t-test: t = 2.28, p = 0.027). However, when restricting the comparison with the five nerve and the 35 tumour samples, no significant difference in mean number of combined methylation sites was observed (mean: 22.2 and 24.8, respectively; unpaired t-test: t = 0.65, p = 0.52). Performing the same analyses on the seven liver, brain, heart and kidney samples revealed a significantly higher mean number of methylation sites in the tumours compared with the tissue samples (mean: 24.8 and 16.3, respectively; unpaired t-test: t = 2.80, p = 0.008). Therefore, tumours have a higher mean number of methylation sites than non-nerve tissues, while the level of methylation is similar between tumours and nerves (table 1).

### Table 1. Comparison of genetic and epigenetic polymorphism in tumour and tissue samples.

<table>
<thead>
<tr>
<th></th>
<th>Tumours (n = 35)</th>
<th>All Tissues (Brain, Heart, Kidney, Liver, Nerve (n = 12)</th>
<th>Nerves (n = 5)</th>
<th>Brain, Heart, Kidney, Liver (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of polymorphic AFLP sites</td>
<td>16</td>
<td>89</td>
<td>83</td>
<td>6</td>
</tr>
<tr>
<td>Mean number of combined methylated sites</td>
<td>24.8</td>
<td>18.8</td>
<td>22.2</td>
<td>16.3</td>
</tr>
<tr>
<td>Mean number of fully methylated sites</td>
<td>7.9</td>
<td>8.1</td>
<td>12.4</td>
<td>5.0</td>
</tr>
<tr>
<td>Mean number of hemimethylated sites</td>
<td>16.9</td>
<td>10.7</td>
<td>9.8</td>
<td>11.3</td>
</tr>
</tbody>
</table>

*a* Shows a significant difference (p < 0.05) between tumour and tissue samples.

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on November 6, 2017 http://rspb.royalsocietypublishing.org/Downloaded from rspb.royalsocietypublishing.org Proc R Soc B 20121720
(c) Comparison of fully methylated amplified fragment length polymorphism sites between tumours and tissues

No overall significant differences in mean number of fully methylated sites were observed among the 35 tumour and 12 tissue samples (mean: 7.9 and 8.1, respectively; unpaired t-test: \( t_{35} = 2.53, p = 0.015 \)). The five nerve samples showed a significantly lower number in mean hemimethylated sites compared with the 35 tumours (mean: 9.8 and 16.9, respectively; unpaired t-test: \( t_{35} = 2.74, p = 0.026 \)). The non-nerve samples exhibited a significantly lower mean number of hemimethylated sites than those observed in the tumours (mean: 11.3 and 16.9, respectively; unpaired t-test: \( t_{40} = 3.42, p = 0.0016 \); table 1).

(d) Comparison of hemimethylated amplified fragment length polymorphism sites between tumours and tissues

The mean number of hemimethylated sites of the 35 tumours was significantly higher than that of the 12 tissue samples (mean: 16.9 and 10.7, respectively; unpaired t-test: \( t_{35} = 2.53, p = 0.015 \)). The five nerve samples showed a significantly lower number in mean hemimethylated sites compared with the 35 tumours (mean: 9.8 and 16.9, respectively; unpaired t-test: \( t_{35} = 2.74, p = 0.026 \)). The non-nerve samples exhibited a significantly lower mean number of hemimethylated sites than those observed in the tumours (mean: 11.3 and 16.9, respectively; unpaired t-test: \( t_{40} = 3.42, p = 0.0016 \); table 1).

(e) Spatial and strain-specific variation in the genetic structure and methylation patterns of tumours

Hierarchical analyses of molecular variance (AMOVA) and minimum spanning networks did not reveal any significant genetic or epigenetic population structure among tumours from different geographical locations (\( p = 0.7 \) and 0.14; figure 1). Furthermore, an exact test of sample differentiation based on genetic and epigenetic haplotype frequencies did not reveal any significant difference among tumours (\( p = 1.0; 30 000 \) Markov steps in both analyses).

We did not observe any significant differences in mean number of combined methylation sites or fully or hemimethylated sites between tumour samples collected from the three geographical regions (one-factor ANOVA; \( F_{31} = 0.34, p = 0.71, F_{31} = 0.19, p = 0.83 \) and \( F_{31} = 0.47, p = 0.63 \), respectively). Moreover, we did not observe any significant variation among the four strains at combined, fully and hemimethylation sites (one-factor ANOVA; \( F_{29} = 0.82, p = 0.49, F_{29} = 1.22, p = 0.33 \) and \( F_{29} = 1.15, p = 0.34 \)).

(f) Temporal variation in tumour methylation

There is a significant variation in mean number of combined methylation sites in tumour samples collected between 2005 and 2010 (one-factor ANOVA, \( F_{31} = 13.8, p < 0.0001 \)). A post-hoc Tukey-Kramer HSD test did not reveal any difference in methylation in 2005, 2006 and 2007 samples (mean: 28.7, 28.6 and 26.1, respectively), but revealed a significant reduction in methylation by 2010 (mean: 13.3; figure 2).

(g) Gene expression of methylation genes

DNMT1 was upregulated in the tumour samples (\( n = 14 \)) compared with nerve by a factor of 3.89, whereas DNMT3a, MBD2 and MBD4 were not differentially expressed (table 2). When only tumour samples collected from 2007/2008 (\( n = 9 \)) were included in the analyses and compared with gene expressions in the five nerve samples, DNMT1 was upregulated in tumour samples from 2007/2008 by a mean factor of 2.267. DNMT3 and MBD2 were slightly downregulated in tumours by a mean factor of 0.157 and 0.249, respectively. MBD4 was not differentially expressed. When only tumour samples collected in 2010–2012 (\( n = 9 \)) were included in the analyses, only DNMT1 was upregulated in the tumour samples compared with nerves by a factor of 5.258, whereas DNMT3a, MBD2 and MBD4 were not differentially expressed. Detailed gene expression results, including standard errors and 95% CIs are summarized in table 2.

(h) Temporal expression of methylation genes in tumours

Comparison of 2007/2008 with 2010/2012 samples revealed significant temporal changes in gene expression levels in genes associated with methylation. DNMT1, DNMT3, MBD2 and MBD4 were all significantly upregulated in 2010/2012 tumours by a mean factor of 5.90, 12.85, 10.79, 9.40, respectively. No significant differences were observed between strains. Detailed gene expression results, including standard error, and 95% CIs are summarized in table 2.

(i) Correlation between tumour gene expression and methylation

There was no difference between gene expression of genes associated with methylation (DNMT1, DNMT3, MBD2 and
MBD4) and any of the three methylation levels (combined, fully and hemimethylation levels; Spearman Rank correlations: $r$ ranging between 0.45 and $-0.02$, $p$ ranging between 0.19 and 0.96).

4. Discussion

Devil Facial Tumour Disease provides a unique opportunity to study cancer evolution in vivo, as the clonal cells pass through different hosts and are exposed to different genetic backgrounds and tumour micro-environments. At the genetic level, DFT cells are remarkably stable [17,18], yet chromosomal strains have recently been described [19]. Our results provide additional support for the clonal origin of the tumour from a Schwann-cell or Schwann cell precursor [10], as we demonstrate that overall methylation patterns in tumours and nerves are remarkably similar, but significantly higher than in other tissues. The observed epigenetic variation did not correspond to the appearance of novel tumour chromosomal strains.

Devil Facial Tumour samples, however, possess significantly higher levels of hemimethylated sites compared with nerves. High levels of hemimethylated CpG dyads and loss of genome-wide methylation are a feature of early cancer development [45,46]. However, DNA hypomethylation can also occur late in cancer progression corresponding to metastatic stages, and to cancer with poor prognosis [45,47]. DNMT1, the principal enzyme responsible for maintaining

Table 2. Relative gene expression results (iterations = 5000). (up, upregulated; down, downregulated; NDE, not differentially expressed.)

<table>
<thead>
<tr>
<th>gene</th>
<th>reaction efficiency</th>
<th>expression</th>
<th>s.e.</th>
<th>95% CI</th>
<th>$p &lt; 0.05$</th>
<th>result</th>
</tr>
</thead>
<tbody>
<tr>
<td>tumour samples (2007 – 2012) versus nerve samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNMT1</td>
<td>0.96</td>
<td>3.89</td>
<td>1.59 – 7.38</td>
<td>1.19 – 51.39</td>
<td>0.005</td>
<td>up</td>
</tr>
<tr>
<td>DNMT3</td>
<td>1.0</td>
<td>0.45</td>
<td>0.15 – 1.24</td>
<td>0.12 – 7.63</td>
<td>0.147</td>
<td>NDE</td>
</tr>
<tr>
<td>MBD2</td>
<td>1.0</td>
<td>0.63</td>
<td>0.213 – 2.00</td>
<td>0.12 – 4.52</td>
<td>0.319</td>
<td>NDE</td>
</tr>
<tr>
<td>MBD4</td>
<td>1.0</td>
<td>1.33</td>
<td>0.48 – 4.09</td>
<td>0.22 – 20.27</td>
<td>0.607</td>
<td>NDE</td>
</tr>
<tr>
<td>tumour samples collected in 2007/2008 versus nerve samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNMT1</td>
<td>0.96</td>
<td>2.27</td>
<td>1.27 – 3.79</td>
<td>1.09 – 6.19</td>
<td>0.003</td>
<td>up</td>
</tr>
<tr>
<td>DNMT3</td>
<td>1.0</td>
<td>0.16</td>
<td>0.16 – 0.21</td>
<td>0.11 – 0.25</td>
<td>0.002</td>
<td>down</td>
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<tr>
<td>MBD2</td>
<td>1.0</td>
<td>0.25</td>
<td>0.15 – 0.43</td>
<td>0.09 – 0.69</td>
<td>0.000</td>
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</tr>
<tr>
<td>MBD4</td>
<td>1.0</td>
<td>0.58</td>
<td>0.34 – 1.17</td>
<td>0.18 – 1.89</td>
<td>0.131</td>
<td>NDE</td>
</tr>
<tr>
<td>tumour samples collected in 2010/2012 versus nerve samples</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>DNMT1</td>
<td>0.96</td>
<td>5.26</td>
<td>2.18 – 10.65</td>
<td>1.39 – 63.27</td>
<td>0.003</td>
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<tr>
<td>DNMT3</td>
<td>1.0</td>
<td>0.79</td>
<td>0.29 – 1.72</td>
<td>0.20 – 7.76</td>
<td>0.667</td>
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<tr>
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<td>1.06</td>
<td>0.47 – 2.46</td>
<td>0.23 – 5.05</td>
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<tr>
<td>MBD4</td>
<td>1.0</td>
<td>2.12</td>
<td>0.63 – 6.59</td>
<td>0.46 – 23.99</td>
<td>0.195</td>
<td>NDE</td>
</tr>
<tr>
<td>tumour samples collected in 2010/2012 versus tumours from 2007/2008</td>
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<tr>
<td>DNMT1</td>
<td>0.96</td>
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<td>0.80 – 43.84</td>
<td>0.001</td>
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<tr>
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<td>12.65</td>
<td>4.35 – 30.71</td>
<td>2.46 – 55.20</td>
<td>0</td>
<td>up</td>
</tr>
<tr>
<td>MBD2</td>
<td>1.0</td>
<td>10.79</td>
<td>3.81 – 28.84</td>
<td>1.97 – 60.09</td>
<td>0</td>
<td>up</td>
</tr>
<tr>
<td>MBD4</td>
<td>1.0</td>
<td>9.40</td>
<td>2.28 – 39.13</td>
<td>1.53 – 159.16</td>
<td>0.001</td>
<td>up</td>
</tr>
</tbody>
</table>

strain 1 tumour samples versus strain 2 tumours

| DNMT1      | 0.96                | 2.253      | 1.48 – 4.13 | 0.97 – 4.66  | 0.139      | NDE    |
| DNMT3      | 1.0                 | 1.586      | 0.68 – 4.97 | 0.32 – 7.71  | 0.528      | NDE    |
| MBD2       | 1.0                 | 1.048      | 0.29 – 2.93 | 0.24 – 5.80  | 0.932      | NDE    |
| MBD4       | 1.0                 | 1.952      | 0.86 – 4.74 | 0.69 – 5.65  | 0.264      | NDE    |

strain 1 tumour samples versus strain 3 tumours

| DNMT1      | 0.96                | 1.882      | 0.68 – 9.51 | 0.52 – 25.82 | 0.628      | NDE    |
| DNMT3      | 1.0                 | 0.948      | 0.16 – 6.23 | 0.15 – 27.77 | 0.960      | NDE    |
| MBD2       | 1.0                 | 0.716      | 0.17 – 2.51 | 0.10 – 10.72 | 0.775      | NDE    |
| MBD4       | 1.0                 | 0.989      | 0.36 – 10.99 | 0.23 – 20.93 | 0.975      | NDE    |

strain 2 tumour samples versus strain 3 tumours

| DNMT1      | 0.96                | 0.836      | 0.25 – 6.03 | 0.22 – 13.83 | 0.849      | NDE    |
| DNMT3      | 1.0                 | 0.598      | 0.11 – 4.39 | 0.08 – 24.43 | 0.608      | NDE    |
| MBD2       | 1.0                 | 0.684      | 0.17 – 2.06 | 0.09 – 10.48 | 0.611      | NDE    |
| MBD4       | 1.0                 | 0.507      | 0.11 – 3.67 | 0.07 – 20.47 | 0.524      | NDE    |
CpG methylation [48,49], is upregulated in DFTs compared with nerves, suggesting methylation maintenance is functional. Therefore, we propose that active demethylation is occurring within the tumour possibly driven by increased expression of MBDB and MBDB genes, coding for proteins with a role in active demethylation. It appears that DFT underwent early hypomethylation when it first arose and that loss of methylation is still occurring 16 years later suggesting that the cancer may be evolving towards a more aggressive form with higher proliferative potential.

Cancer is an evolutionary process [50–52], with a mosaic of cells competing for space and resources. Variants best able to evade detection by the immune system and adapt and survive have evolutionary benefits [50]. Our results suggest that DFTD is evolving via epigenetic modifications and that plasticity in methylation patterns could lead to changes in gene expression over time. Individual DFT cells can undergo independent evolutionary changes, fuelling clonal evolution [8,17,19].

Recent field data provides anecdotal support for the phenotypic evolution of DFT clones. Low prevalence of DFTD has been observed in West Pencil Pine, a population in northwestern Tasmania. The disease has been in the area for over 6 years [53,54], yet only 20 per cent of the population is affected. This difference could be either owing to differences in the hosts or differences in the tumours. We recently showed that there was no observable difference in MHC patterns of diseased and healthy devils and are currently looking genome-wide [55]. But it is also possible that DFT variants in the region differ to those in the east owing to epigenetic modifications. Hypermethylation of genes responsible for cell cycle arrest or immune evasion, or hypomethylation of transposable elements and genes involved in cell adhesion could potentially provide DFTD with evolutionary benefits leading to the development of novel disease phenotypes.

There is only one other naturally occurring transmissible cancer, canine transmissible venereal tumour (CTVT). CTVT is believed to have evolved in inbred wolves that lacked MHC diversity (reviewed in [12]) and then over time evolved immune evasion strategies allowing it to cross the MHC barrier and infect MHC disparate canids by modulation of cell-surface MHC expression [56]. It has been proposed that in CTVT, MHC expression is modulated by epigenetics as it is in various human tumour cell types [57,58]. It is worth noting that recent skin graft experiments show that skin grafts between MHC-similar animals are rejected, albeit more slowly, and that T-lymphocyte infiltration drives this rejection [59]. There is no evidence of T-lymphocyte infiltration into DFTs, indicating that the tumour may be capable of immune evasion or immune suppression. The role of epigenetics in immune evasion should be explored.

An unexpected outcome of this study was the observation of high levels of genomic AFLP polymorphism in the five nerve samples compared with tissues taken from the same individuals. Neuronal genetic mosaicism has been suggested to be the result of the activity of transposable elements during brain development [48,60], aneuploidy (a normal feature of the human and mouse central nervous system) [49] and/or from genomic copy number variations in humans [61]. This warrants further attention and may provide insights into the mechanisms that led to the emergence of DFTD.

In summary, we present, to our knowledge, the first evidence of DNA methylation (epigenetics) in DFTD evolution showing that the cancer, despite being genetically stable, is a dynamically changing entity with a high phenotypic plasticity and evolutionary potential. The observed temporal methylation and gene expression changes suggest that this clonally transmissible cancer is capable of adapting to its micro-environment via epigenetic alterations. Future studies should focus on the role of epigenetics in DFTD evolution, and the effect epimutations have on disease phenotypes. Understanding the role of epimutations in the evolution of this parasitic cancer will be relevant to other cancers that evolve and die within a single host, and may provide unique insights into cancer progression.

The research was carried out with approval from the DPIPWE (Tasmania) animal ethics committee, Animal Ethics no. 101 2010/11.

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