Exploration of the mutational landscape of cutaneous leiomyoma confirms *FH* as a driver gene and identifies targeting purine metabolism as a potential therapeutic strategy

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Dear Editor, Treatment of cutaneous leiomyoma (cLM) is based on the number or anatomical location of the lesions, and the degree of discomfort they cause. Surgical or ablative procedures can remove the lesions, however, recurrence is an issue. Such procedures are also not always suitable for patients with numerous lesions, such as those with hereditary leiomyomatosis and renal cell cancer (HLRCC), a familial condition caused by germline variants in the *Fumarate hydratase* (*FH*) gene. *FH* encodes the enzyme fumarase, which is part of the citric acid cycle and helps convert fumarate to malate.

In this era of precision medicine, comprehensive knowledge of driver events is essential for the development of new cancer treatments. Thus, to comprehensively explore the mutational landscape of these tumours and identify candidate driver events, we performed a retrospective, multi-institutional, whole-exome sequencing and RNA sequencing study on a large cohort of patients with cLM. The samples included 53 formalin-fixed, paraffin embedded cases of cLM from 37 patients, including seven patients with two to five independent lesions. The sequencing data have been deposited in the European Genome and Phenome Archive.

DNA extracted from 42 cLM and perilesional normal tissue samples were analysed by whole-exome sequencing. cLM showed a low tumour mutational burden, with a median of 0.12 mutations/megabase. Analysis of both somatic and germline genetic alterations found in cLM highlighted loss of the *FH* tumour suppressor gene as a key driver event, as shown in Figure 1a. Recurrent somatic mutation of *FH* was found to be statistically significant (q-value = 0.0049, dNdScv algorithm),[1] with somatic mutations found in four of 42 (9.5%) cLM samples. Sequencing of the normal tissue identified germline variants in *FH* in 13 of 27 (48%) patients, with eight of 13 (62%) of the variants reported in ClinVar as 'pathogenic' or 'likely pathogenic'. In these 27 patients, *FH* was biallelically inactivated in 16 tumours (from

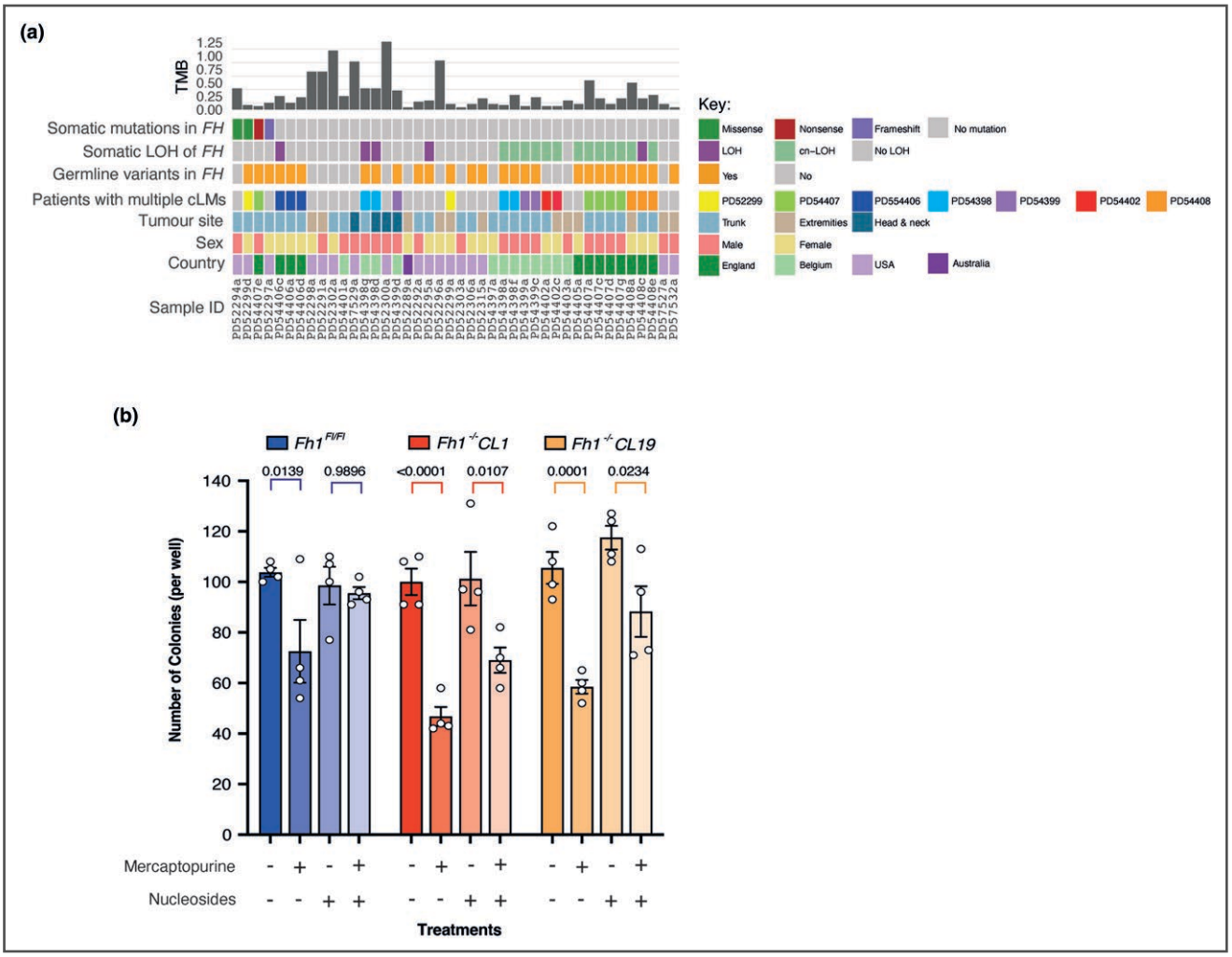

Figure 1 The importance of the *FH* tumour suppressor gene in cutaneous leiomyoma. (a) Oncoplot highlighting the various ways *FH* is genetically altered in 42 cutaneous leiomyoma (cLM) samples from 27 patients. (b) The effect of mercaptopurine in *FH*-proficient and *FH*-deficient cells. The results of the clonogenic assay clearly demonstrate that addition of nucleosides can rescue the growth inhibitory effects induced by mercaptopurine, but only in *FH*-proficient cells (*Fh1*$^{fl/fl}$), not *FH*-deficient cells (*Fh1*$^{-/-}$*CL1* and *Fh1*$^{-/-}$*CL19*). Statistical analysis was performed using a two-way ANOVA with Tukey multiple comparisons test (n=4). cn-LOH, copy-neutral LOH; LOH, loss of heterozygosity; TMB, tumour mutational burden (shown as mutations/megabase)

six patients), as they harboured either somatic loss of heterozygosity (LOH) or somatic copy-neutral LOH in addition to germline *FH* variants. Additionally, three tumours with germline *FH* variants did not harbour copy-number loss of *FH*, but somatic *FH* mutations, possibly resulting in biallelic inactivation. Importantly, the recurrent somatic deletion encompassing *FH* was statistically significant (q-value = 1.35×10^{-6}; GISTIC2.0 algorithm),[2] implicating LOH of *FH* as a driver event. Whole-exome analysis did not identify other driver genes; after *FH* the most frequently mutated genes were found in two of 42 (4.8%) samples (with the exception of one gene that was mutated in three samples, all from the same patient). However, these genes were not significantly mutated above the background mutation rate, and thus are unlikely to be candidate driver genes. In summary, we have confirmed that a large proportion of patients with cLM have germline *FH* variants and additionally show that somatic alteration of *FH* also drives cLM, with biallelic inactivation of *FH* being a frequent event in cLM.

RNA from 26 cases of cLM were analysed by pull-down RNA sequencing to identify fusion genes, as they represent potential driver events. Focusing on in-frame and frameshift transcripts, four different fusion genes were identified in the cLM cohort. *MYLK::MAP3K2* was found in two samples, and has not been reported previously in any tumour type. In both cases, the breakpoints were identical and the resulting transcript containing all but the final exon of *MYLK* spliced to exons 10–13 of *MAP3K2*. The other three fusions identified in the cLM cohort (*YAP::LPP*, *STXBP1::MAP1B* and *FN1::FGFR2*) were found in only one sample each. They also have not been reported previously in any tumour type. Therefore, fusion genes do not appear to be major drivers of cLM.

We next interrogated data from the Cancer Dependency Map[3] (https://depmap.org/portal) to identify a list of *FH* 'co-essential genes', denoting genes essential in cancer cell lines that also require *FH* for survival. Gene-set enrichment analysis was performed to identify classes of

overrepresented genes, and enrichment of the citric acid cycle and purine metabolism pathways was found. All 12 genes we identified in these pathways were expressed in the cLM cases (by assessment of the RNA sequencing data). Importantly, these pathways overlap at one molecule – fumarate, the metabolite known to accumulate in *FH*-deficient cells.[4] Treatment of *Fh1*-proficient and -deficient cell lines[5] with the purine antagonist and chemotherapeutic agent, mercaptopurine, significantly decreased the growth/colony formation of all cell lines, as shown in Figure 1b. However, the addition of nucleosides was able to rescue only the *Fh1*-proficient cells, consistent with defective purine salvage in *FH1*-deficient cells.[6]

This suggests purine metabolism is a targetable vulnerability for *FH*-deficient cLMs. As mercaptopurine is already used off-label for various dermatological conditions,[7] and a hydrogel formulation of mercaptopurine has been described recently,[8] it is tempting to speculate that this may serve as a promising localized drug delivery system for the subset of patients having extensive *FH*-mutant cLMs. The addition of nucleosides would be beneficial, as we showed that their presence can minimize the toxicity of mercaptopurine on *Fh* wild-type cells (Figure 1b).

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Conflicts of interest: The authors have no competing or financial interests to declare.

Data availability: The sequencing data have been deposited in the European Genome and Phenome Archive under the accessions EGAD00001014787 (whole-exome sequencing) and EGAD00001014788 (RNA sequencing).

Ethics statement: Ethical approval for the use of all patient samples in this project was obtained by a local committee at the institution of origin and the Research Governance at the Wellcome Sanger Institute. This study is part of the DERMATLAS Project that has been approved by the NHS Health Research Authority; Research Ethics Committee (REC) reference: 21/PR/1024, IRAS project ID: 304621.

Patient consent: Not applicable.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Appendix S1 Full list of authors and affiliations.

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