to lower levels of a proliferation-inhibiting protein known as transforming growth factor-β and enhanced expression of the protein cyclin D1, culminating in accelerated hepatocyte proliferation. Then, during the angiogenic phase, Ang2 levels recover and stimulate the proliferation of LSECs in a process that depends on the vascular endothelial growth factor receptor 2 (VEGFR2) and on expression of the protein Wnt2.

The authors also show that Ang2 plays an important part in the response to chronic chemical injury following exposure of the liver to the toxin carbon tetrachloride. Thus, it seems that LSECs first act 'altruistically', to enhance hepatocyte recovery, and later boost their own growth. Interestingly, a report[3] measuring Ang2 levels in patients with acute liver failure revealed that higher levels are associated with a worse clinical outcome, further documenting the clinical and therapeutic relevance of Ang2 signalling and of the blood vessels that produce this protein.

An earlier study[4] had already demonstrated the importance of LSECs and VEGFR2 in liver repair, although in that work VEGFR2 was implicated in the inductive phase by mediating the expression of the proteins Id1, Wnt2 and hepatocyte growth factor (HGF), which contribute to the induction of hepatocyte proliferation. The same research group also showed[5] that angiocrine signalling controls the balance between optimal organ repair after acute injury and scarring after prolonged insult — expression of the receptor protein CXCR7 in LSECs is responsible for the pro-proliferative Id1–Wnt2/HGF response after acute injury. By contrast, CXCR7 expression is suppressed during chronic injury, leading to defective repair and scarring.

Future studies using cell-specific inactivation of Ang2 and VEGFR2 may help to clarify the complex interplay of these factors during the inductive and angiogenic phases of liver regeneration. However, Hu and colleagues' findings clearly show that Ang2 expression orchestrates the proliferation of hepatocytes and LSECs during this process. The angiogenic action of Ang2 is consistent with the protein's reported role in protecting blood vessels against stress[6]. Other angiocrine factors produced by the endothelium have been identified as contributors to liver regeneration, including prostaglandin E2 (ref. 7), epoxyeicosatrienoic acids[8] and nitric oxide[9]. The involvement of endothelial cells and their signals has also been demonstrated for proper regeneration of the lung[8,10], haematopoietic stem cells[11] and the kidney[8].

The liver responds swiftly to organ injury. But is it plausible that liver endothelial cells initiate and orchestrate this response? It seems so. After partial hepatectomy, the remaining liver tissue is intact and uninjured, and is not exposed to toxins or dying hepatocytes. By contrast, the vasculature is subject to immediate changes in blood flow and thus potentially to altered exposure to soluble signalling factors. Because the entire blood flow entering the liver circulates through a much reduced liver-cell mass, physical blood-flow parameters or soluble-factor concentrations will also change; this could represent the initial signal of injury immediately sensed by LSECs[1]. Similarly, liver injury by toxins leads to cell swelling and cell death, which alter blood-flow dynamics. Endothelial cells are therefore extremely well equipped to both sense and immediately respond to changes in the integrity, size and metabolic capacity of the liver.

One could also imagine a direct role for endothelial cells in promoting the proliferation of other liver-cell types, such as biliary epithelial cells or stellate cells, and in the cessation of proliferation once hepatic repair is complete. Is a single molecule responsible for regulating the entire process? Probably not, because optimal liver regeneration after injury is essential for the survival of the organism, and a multitude of factors and signals are known to act in concert, and redundantly, to achieve rapid and efficient functioning of the organ. Hu and colleagues' work, however, demonstrates that signals emerging from the hepatic vasculature are dynamically modulated to govern the entire temporal sequence of hepatic repair. What can we learn from this? That to promote hepatic regeneration in our sickest patients, we need to take the brakes off as much as we need to press the accelerator. ■

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CANCER

Persistence of leukaemic ancestors

The early development of acute leukaemias is assumed for the most part to be clinically silent and transient. But it now seems that ancestral precancerous cells are identifiable and persistent. SEE ARTICLE P.328

NICOLA E. POTTER & MEL GREAVES

Aggressive leukaemias often present clinically out of the blue, without previous indications of cancer. But evolutionary models of cancer development posit a time-ordered, stepwise process involving the accumulation of mutations, proliferation of mutated cells into expanded clonal populations and selection of the fittest cells[1]. Such models imply that any seemingly sudden case of cancer will have arisen from 'silent' precursor cells that have no clinical impact. In this issue, Shlush *et al.*[2] (page 328) provide compelling evidence that the early-stage cells of acute myeloid leukaemia are not completely outcompeted and rendered extinct by their more aggressively cancerous and numerous progeny, but instead persist and show defined genetic and functional properties.

The cancerous myeloid cells (a subset of white blood cells) of patients with acute myeloid leukaemia (AML) often have a mutation in the gene encoding the DNA-methyltransferase enzyme DNMT3a. In two studies of patients with *DNMT3a*-mutated AML — one involving 4 patients and the other 17 — Shlush and colleagues made the unexpected observation that, in 15 patients, the *DNMT3a* gene carried the same mutation at a low rate in T cells (a type of immune cell that belongs to the lymphoid system) in the blood. Strikingly, however, the T cells lacked other alterations present in the leukaemic cells of the same patients, including mutations in the gene *NPM1*. The *DNMT3a* mutation was also detected at variable frequency in other immune cells (B and NK cells) at the time of AML diagnosis. Finding this mutation in non-myeloid cells hinted at its occurrence in a precursor cell that gives rise to blood cells of both the lymphoid and myeloid lineages.

The frequency with which *DNMT3a* and *NPM1* were mutated in leukaemic cells was

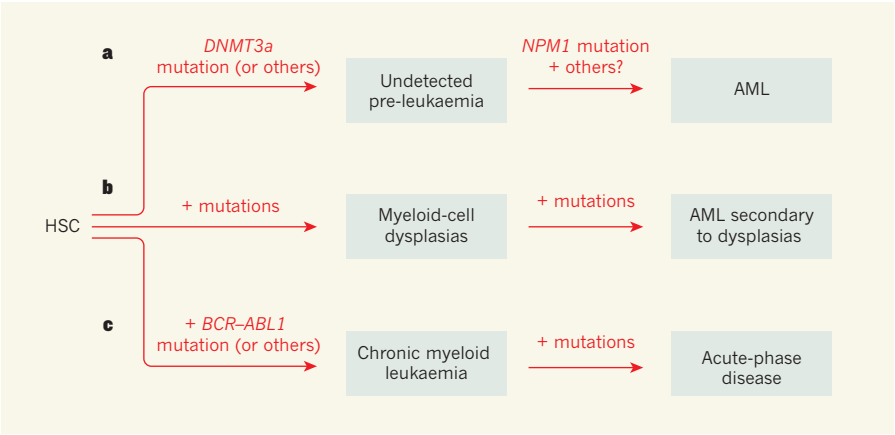

Figure 1 | Different routes to acute myeloid leukaemia. Cancer occurs when cells accumulate mutations over time. In acute myeloid leukaemia (AML), the first cell to be transformed to a cancer-like state is typically a haematopoietic stem cell (HSC). Differentiation of this cell can lead to AML through one of three intermediate stages. **a**, Shlush *et al.*[2] found that, when AML presents in the clinic with no warning, the cancer-initiating mutation in the HSC is often in the gene *DNMT3a*. Such precancerous cells are not clinically detectable. Further mutations, for example in the gene *NPM1*, then lead to AML. **b**, AML can also arise as a secondary event to myeloid-cell dysplasias, in which mature myeloid cells are not generated effectively. Subtypes of myeloid-cell dysplasias can arise from different subsets of mutations. **c**, Alternatively, further mutations in cells of chronic myeloid leukaemia (which already carry a mutation in *BCR–ABL1* or other genes) can result in more aggressive, acute-phase disease.

equally high in all but two patients studied. This suggests that both mutations were probably present in a 'founder' clone population, from which the leukaemic population expanded. From this starting point, the authors astutely deduced that the first cell to acquire a leukaemic 'driver' mutation in *DNMT3a* in these patients was a haematopoietic stem cell (HSC) — the precursors of the lymphoid and myeloid lineages — and that descendants of this cell persisted as an expanded, competitive clonal population (Fig. 1). However, whether mutation of *DNMT3a* is by itself sufficient to both initiate and sustain the growth of a clonal population before the onset of leukaemia is uncertain, because Shlush and colleagues analysed only specific genes, and so the mutational complexity of the cancers was probably underestimated[3]. Although mutation of *NPM1* can initiate AML in mouse models of the disease[4], and is often seen in humans with AML, it seems to be a secondary alteration in most cases of AML that carry a *DNMT3a* mutation.

These data agree with the results of functional experiments[5] indicating that DNMT3a normally promotes the differentiation of HSCs into other cell lineages at the expense of the HSCs' self-renewal. The mutation in *DNMT3a* in AML results in a loss of the enzyme's catalytic activity[6]. But the mutated protein can repress the function of the normal protein encoded by the non-mutated copy of *DNMT3a*, and this is expected to increase self-renewal[7]. Shlush *et al.* found mutant *DNMT3a* in a relatively high percentage of HSCs from each AML patient (up to 30%), suggesting a high degree of competitive self-renewal of the *DNMT3a*-mutant cells. However, they also observed some normal differentiation of the *DNMT3a*-mutant HSCs into lymphoid and myeloid cell lineages.

The authors confirmed this functionally by repopulating immune-cell-deficient mice with blood cells from two patients with AML. In around 75% of these mice, multiple cell lineages were established. Ten of 12 such mice were studied further, and were found to have a high frequency of the *DNMT3a* mutation but to have normal *NPM1*, indicating a functional dominance of the *DNMT3a*-mutated HSC population. Only a minority of the mice had a myeloid leukaemic population with mutations in both *DNMT3a* and *NPM1*.

Shlush and co-workers further showed that *DNMT3a*-mutant HSCs and their differentiated progeny persisted in patients' blood even when AML was in remission following chemotherapy, indicating that at least some of these pre-leukaemic ancestral cells were resistant to treatment. This may be because a high fraction is quiescent — as in other types of leukaemia and cancer. Collectively, the data highlight the persistence of benign pre-leukaemic clones. Other data point to a similar scenario, including the observation[8] of another mutation often found in AML, the *ETO–RUNX1* fusion, in HSCs and B cells. A further study[9] reported the identification of ostensibly normal HSCs, myeloid and lymphoid cells that had a subset of the mutations present in the immature AML myeloid cells of the same patient. Interestingly, and in contrast to Shlush and colleagues' findings, that study provided evidence of a linear, or evolutionary, order of several mutations in the HSC-derived pre-leukaemic cells.

More generally, these studies[2,8,9] contribute to an emerging portrait of the complexity of clonal evolutionary pathways that lead to AML[3,10,11] (Fig. 1). The premalignant phase, arising predominantly in HSCs, can be highly variable, both clinically and biologically, depending on the mutations, or combination of mutations, involved[11] and their functional impact. Common secondary mutations, such as those in *NPM1*, may result in more stringent arrest of differentiation, or more vigorous cell proliferation — steps that lead to acute-phase disease. Secondary mutations, in contrast to the founder (or very early) alterations, often seem to arise in more-lineage-committed myeloid progenitors, as demonstrated for *NPM1* by Shlush *et al.*, and previously in chronic myeloid leukaemia[12]. A study[13] of acute lymphoblastic leukaemia has similarly suggested that secondary mutations arise in more-differentiated progeny cells, and this might be expected to be a feature of other types of cancer.

As Shlush and colleagues point out, their data have several clinical implications. One is that, as a persistent and likely founder, the *DNMT3a* mutation could be both a therapeutic target and a marker for tracking residual disease. By contrast, therapies targeting secondary mutations, such as *NPM1*, will probably have only transient success. Another possibility suggested by the persistence of the pre-leukaemic clones and of mutant HSCs in remission is that these cells provide a cellular reservoir for relapse. The observation that some patients who present with *DNMT3a–NPM1* double-mutant AML but, following treatment, relapse with cancers that retain only the *DNMT3a* mutation, is compatible with this idea[14]. Finally, it would be of interest to determine how often *DNMT3a*-mutant clones arise from HSCs in ageing adults and, where such clones emerge, how frequently the evolutionary transition to AML occurs, and over what time frame. ■

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