Forever Young

Geneticists use Pathway Commons data to link gene implicated in leukemia to well-studied oxidative-stress pathways that maintain stem-cell renewal

DNA-damage-induced differentiation of leukemic cells as an anti-cancer barrier
Santos *et al.* Nature Volume 514, Issue 7520, 107-111 (2 October 2014)

Quick Summary

- MLL4 promotes stem-cell renewal in normal hematopoietic cells and maintenance of leukemia induced by MLL-AF9 oncogene
- Oxidative stress known to trigger differentiation at the expense of stem-cell renewal
- Pathway Commons data linked *MLL4* to oxidative stress detoxification pathways

Author Profile

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Context

A deep connection has been forged between aging, the ability to replace and repair tissue over time and DNA damage. Much attention has been directed towards stem-cells – those rare entities that give rise to multi-potent daughters alongside those destined to replenish tissues through terminal differentiation[1]. Stem-cells have spurred much interest for their therapeutic potential and participation in oncogenesis.

Question

What factors determine blood stem-cell renewal and differentiation in normal and pathological circumstances?

Researcher Goals

The Mixed Lineage Leukemia (*MLL*) gene family encode histone methyltransferases and the founding member, *MLL1,* was identified in a subset of acute infant leukemias with poor prognosis. A translocation fusing *MLL1* with *AF9* is associated with acute myeloid leukemia (AML) in infants and the analogous fusion in mice (MLL-AF9) recapitulates the disease[3]. *MLL4* is of particular clinical interest as the most frequently altered tumor suppressor in non-Hodgkin's lymphoma (NHL)[4]. What constitutes MLL4 tumor suppressor activity is unclear.

Santos *et al.* [2] combine classic genetic and cell biology approaches with pathway analysis to present a unified, mechanistic picture of how *MLL4* influences hematopoietic stem-cell (HSC) renewal in normal and leukemic cells.

> *"Pathway analysis represents an approach to simplify and power the interpretation of differential gene expression assays"*

Approach

To better understand the role of *MLL4*, Santos *et al.* generated mice deficient for *MLL4* and examined blood for changes in hematopoietic stem-cells (HSC). In previous studies, imposing 'stressful' conditions was key to revealing the consequences of knockouts. For hematopoietic cells this means driving them into the cell cycle through growth factor stimulation *in vitro* or competitive reconstitution of bone marrow *in vivo*. In this case, *MLL4* mutants had fewer HSC and more differentiated myeloid cells, supported by the observations that HSC division was biased towards producing differentiated myeloid cells (symmetric commitment) at the expense of multipotent daughters (self-renewal). The effects of *MLL4* on renewal mirrored the effects of *MLL1* removal[5]. How might *MLL4* tip the balance away from differentiation and back towards renewal?

> *"The observed a drop in glutathione-detoxification pathway (from Pathway Commons) and Forkhead box O transcription factors (FoxO) which led investigators to posit the more general picture that MLL4 loss led to a general disruption of protection from oxidative stress."*

Pathway analysis represents an approach to simplify and power the interpretation of differential gene expression assays. One one hand, it simplifies analysis by grouping potentially long lists of individual genes into their respective pathways. On the other hand, it provides greater power to

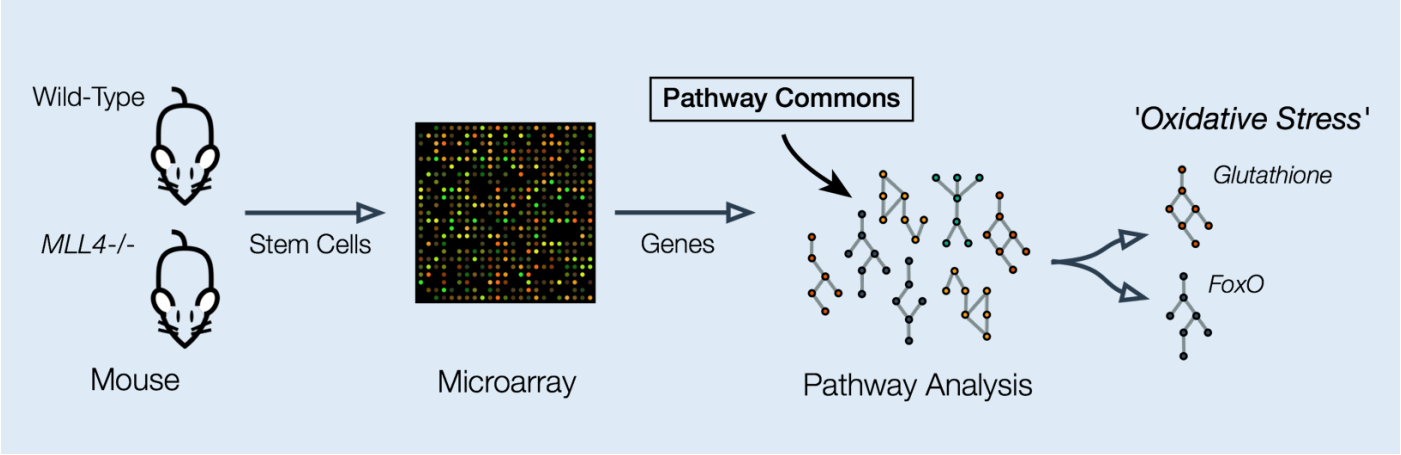

Figure 1. Pathway analysis unifies MLL4 and protection from oxidative stress. Stem cells from wild-type and MLL4-knockouts were analyzed by microarray analysis. The resulting lists of gene expression differences were analyzed by gene set enrichment analysis (GSEA) using pre-defined pathways from Pathway Commons, Gene Ontology and Reactome. Oxidative stress pathways have a pivotal role in avoiding DNA-damage induced differentiation.

detect alterations in pathways using changes in gene expression, despite the fact that any single gene might not necessarily be flagged as statistical significant on its own.

Santos *et al.* turned to pathway analysis to peer into the pathways affected by MLL4 loss (Fig. 1). In particular, gene set expression analysis (GSEA) was applied to microarray data from wild-type vs *MLL4* knockout HSC. The observed a drop in glutathione-detoxification pathway (from Pathway Commons) and Forkhead box O transcription factors (FoxO) which led investigators to posit the more general picture that *MLL4* loss led to a general disruption of protection from oxidative stress. Indeed, this directly prompted follow-up experiments revealing an increase in reactive oxygen species (ROS) and DNA damage in *MLL4* mutants.

Pathway analysis cast a light on the mechanisms affected by *MLL*, and in doing so, allowed the authors to tap into a rich body of knowledge surrounding ROS, DNA damage, HSC, and leukemia. Oxidative stress has long been known to be an agent of aging by reducing stem-cell renewal in stress conditions such as cell cycle entry and bone marrow reconstitution [6].

Armed with this knowledge, Santos *et al.* set about to provide a link between *MLL4,* oxidative stress pathways, and leukemia. First, MLL-AF9 required *MLL4* to cause leukemia, similar to the requirement for *FoxO*[7]. Second, loss of *MLL4* resulted in a noticeable shift of AML cells towards myeloid differentiation and away from renewal. Third, another application of pathway analysis, this time examining how loss of *MLL4* affects MLL-AF9 cells once again revealed changes to oxidative stress through *FoxO* genes and the glutathione-detoxification pathway. Finally, adding-back FOXOs in MLL-AF9 compensated for the defects associated with *MLL4* loss. Altogether, this supported a picture whereby MLL4 mitigates genotoxic stress and thereby biases stem-cells away from terminal differentiation.

Summary

Pathway Commons provides a single point of access for GSEA formatted gene sets (.gmt) from a variety of data sources. Santos *et al.* successfully incorporated PC data into their GSEA workflow to uncover a link between MLL activity and DNA-damage-mediated stem cell renewal in normal and leukemic cells.

References

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