MICROENVIRONMENT

Dependency

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Several solid tumours depend on intrinsic hedgehog (Hh) pathway activation for survival. Hh ligands are also expressed in all lymphoid organs and are necessary for survival of germinal centre B cells, from which some B-cell lymphomas arise. Christine Dierks *et al.* now

show that the survival of B-cell lymphomas depends on extrinsic Hh signalling from the stroma.

The authors were able to grow lymphoma cells taken from the well-established *Eμ-Myc* mouse model for lymphoma with media conditioned by stromal cells isolated from diseased animals. This showed that soluble factors secreted by stromal cells contribute to the survival of the lymphoma cells. Different growth factors were tested for their ability to support lymphoma growth after the withdrawal of conditioned media, and although various cytokines provided little stimulation, the addition of the recombinant Hh ligands *SHH* or *IHH* increased the number of viable cells about threefold.

Inhibition of the Hh pathway with cyclopamine or an Hh-neutralizing antibody decreased survival of the *Eμ-Myc*-lymphoma cultures. The authors confirmed that Hh signalling also has a role in human non-Hodgkin lymphoma and multiple myeloma, as the survival of patient tumour cells cultivated on stromal cells that express *IHH* also decreased on treatment with cyclopamine. Inhibition of Hh led to decreased transcription of Hh target genes and induction of apoptosis in the *Eμ-Myc* lymphomas within 24 hours. In addition, when luciferase-expressing lymphoma cells were injected into mice and

then treated with cyclopamine, the cyclopamine-treated mice only showed minimal signs of disease, whereas control mice had high luciferase expression in lymph nodes and spleen.

So, how does Hh signalling induce apoptosis in the *Eμ-Myc* lymphoma cells? Experiments with *Eμ-Myc* lymphoma cultures derived from mice null for *Trp53*, *Cdkn2a*, *Casp3*, *Bax* or transgenic for *Bcl2*, showed that apoptotic cell death seen in the lymphoma cells after Hh inhibition is mediated by the classical mitochondrial BCL2 pathway. To confirm this, luciferase-expressing *Eμ-Myc* lymphoma cells transgenic for *Bcl2* or null for *Trp53* were mixed with stromal cells and injected into nude mice. Cyclopamine reduced the expansion of the *Trp53*-null cells but not the *Bcl2*-expressing cells. Cyclopamine also decreased the infiltration of splenic cells and reduced lymphoma mass in mice with established lymphomas.

This paper shows that B-cell malignancies are dependent on Hh signalling from the stroma for survival, and suggests a new avenue for the treatment of both lymphoma and multiple myeloma.

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ORIGINAL RESEARCH PAPER Dierks, C. *et al.* Essential role of stromally induced hedgehog signalling in B-cell malignancies. *Nature Medicine* **13**, 944–951 (2007)

DIGITALSTOCK