Nature Reviews Cancer | AOP, published online 28 March 2013; doi:10.1038/nrc3511

SENESCENCE

ConnectING endocytosis

> “disrupting endocytosis … could induce senescence”

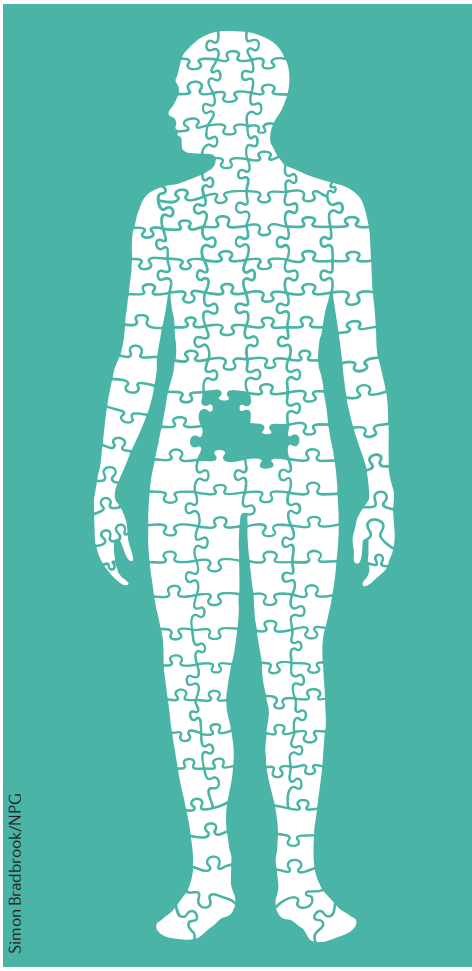
The inhibitor of growth (ING) proteins are epigenetic regulators that have tumour suppressor roles, particularly as mediators of senescence. Expression of the splice variant ING1A induces senescence *in vitro*, but the mechanisms have been poorly characterized. A new study identifies downstream effectors of ING1A-mediated senescence induction, including a role for defective endocytosis.

To characterize the genes that show altered expression in response to ING1A expression, Rajarajacholan, Thalappilly and Riabowol carried out gene expression microarray analyses of Hs68 human fibroblasts overexpressing ectopic ING1A compared with control Hs68 cells. They found endocytosis-associated genes among the most differentially regulated genes, particularly the upregulation of the endocytic assembly factor intersectin 2 (*ITSN2*).

Next, the authors tested the functional links between ING1A, endocytosis and senescence. A common marker for endocytosis is the internalization of epidermal growth factor receptor (EGFR) in response to EGF treatment. Overexpressing ING1A in different cell types *in vitro* inhibited the endocytosis and degradation of EGFR, but this was enhanced in *Ing1*-deficient mouse embryo fibroblasts (MEFs) compared with wild-type MEFs. Therefore, ING1A seems to have a role in regulating endocytosis. Moreover, independently disrupting endocytosis — either pharmacologically by treating with Dynasore or genetically by manipulating the expression of endocytosis proteins — could induce senescence.

To determine whether the ING1A-regulated endocytosis protein ITSN2 is a key mediator of senescence, the authors overexpressed ITSN2 in fibroblasts, which was sufficient to induce some markers of senescence. These included INK4A expression, senescence-associated β-galactosidase activity and senescence-associated heterochromatic foci, although cells did not adopt the characteristic flattened cellular morphology of senescence. Furthermore, ITSN2 knockdown could diminish various features of senescence in ING1A-overexpressing cells — such as cell cycle arrest and E2F target gene silencing — thus providing evidence that ITSN2 is required for some senescence outputs. Chromatin immunoprecipitation experiments revealed that ING1A binds upstream of the *ITSN2* gene, which is consistent with *ITSN2* being a direct transcriptional target of ING1A during senescence induction.

Turning to additional senescence settings, the authors found that endogenous ING1A and ITSN2 were upregulated during replicative senescence and in senescence triggered by the oxidative stress agent tert-butyl hydroperoxide, whereas neither protein was upregulated in senescence induced by the DNA-damaging agent doxorubicin. Overall, this implicates the ING1A–ITSN2 pathway in some, but not all, senescence contexts.



Simon Bradbrook/NPG

It will be interesting to characterize the mechanistic connections between endocytosis and senescence and to assess the contribution of ING1A, ITSN2 and endocytosis disruption to the tumour-suppressive effects of senescence *in vivo*.

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ORIGINAL RESEARCH PAPER Rajarajacholan, U. K., Thalappilly, S. & Riabowol, K. The ING1a tumor suppressor regulates endocytosis to induce cellular senescence via the Rb-E2F pathway. *PLoS Biol.* **11**, e1001502 (2013)