NOTCH-mediated non-cell autonomous regulation of chromatin structure during senescence

Aled J. Parry 1, Matthew Hoare 1,2, Dóra Bihary 3, Robert Hänsel-Hertsch 1, Stephen Smith 4, Kosuke Tomimatsu 1, Elizabeth Mannion 1, Amy Smith 1, Paula D’Santos 1, I. Alasdair Russell 1, Shankar Balasubramanian 1,5, Hiroshi Kimura 6, Shamith A. Samarajiwa 3 & Masashi Narita 1

Senescent cells interact with the surrounding microenvironment achieving diverse functional outcomes. We have recently identified that NOTCH1 can drive ‘lateral induction’ of a unique senescence phenotype in adjacent cells by specifically upregulating the NOTCH ligand JAG1. Here we show that NOTCH signalling can modulate chromatin structure autonomously and non-autonomously. In addition to senescence-associated heterochromatic foci (SAHF), oncogenic RAS-induced senescent (RIS) cells exhibit a massive increase in chromatin accessibility. NOTCH signalling suppresses SAHF and increased chromatin accessibility in this context. Strikingly, NOTCH-induced senescent cells, or cancer cells with high JAG1 expression, drive similar chromatin architectural changes in adjacent cells through cell–cell contact. Mechanistically, we show that NOTCH signalling represses the chromatin architectural protein HMGA1, an association found in multiple human cancers. Thus, HMGA1 is involved not only in SAHF but also in RIS-driven chromatin accessibility. In conclusion, this study identifies that the JAG1–NOTCH–HMGA1 axis mediates the juxtacrine regulation of chromatin architecture.
Cellular senescence is an autonomous tumour-suppressor mechanism that can be triggered by pathophysiological stimuli including replicative exhaustion, exposure to chemotherapeutic drugs and hyper-activation of oncogenes, such as RAS. Persistent cell cycle arrest is accompanied by diverse transcriptional, biochemical and morphological alterations. These senescence hallmarks include increased expression and secretion of soluble factors (senescence-associated secretory phenotype (SASP)) and dramatic alterations to chromatin structure. Importantly, the combination, quantity and quality of these features can vary depending on the type of senescence. Senescent cells have profound non-cell autonomous functionality. The SASP can have either protumorigenic or antitumorigenic effects and act in an autocrine or paracrine fashion. SASP can be triggered by pathophysiological stimuli including replicative exhaustion, exposure to chemotherapeutic drugs and hyper-activation of oncogenes, such as RAS.

The NOTCH signalling pathway is involved in a wide array of developmental and (patho-)physiological processes. NOTCH has roles in differentiation and stem cell fate and perturbations have been linked to tumorigenesis where NOTCH can have either oncogenic or tumour-suppressive functionality. The pathway involves proteolytic cleavage of the NOTCH receptor upon contact-mediated activation by a ligand of the JAGGED (JAG) or DELTA family on the surface of an adjacent cell. The cleaved NOTCH-intracellular domain translocates to the nucleus where, together with transcriptional co-activators such as mastermind-like 1 (MAML1), it drives transcription of canonical target genes, including the HES and HEY family of transcription factors. NOTCH signalling has also been shown to induce a type of senescence, NOTCH-induced senescence (NIS), where cells are characterised by distinct SASP components. Recently, we showed that during NIS there is a dramatic and specific upregulation of JAG1 that can activate NOTCH1 signalling and drive NIS in adjacent cells (‘lateral induction’). During senescence, particularly in oncogenic RAS-induced senescent (RIS) fibroblasts, characteristic changes to chromatin culminate in the formation of senescence-associated heterochromatic foci (SAHFs), layered structures facilitated by spatial

**Fig. 1** NOTCH1 signalling has a chromatin ‘smoothening’ effect that blocks SAHF. **a** Diagram illustrating the NOTCH1 signalling pathway, which can be repressed chemically by using DAPT or genetically by expressing dominant-negative MAML1 (dnMAML1). **b** IMR90 ER:HRASG12V cells were infected with control vector or N1ICD-FLAG and incubated with 100 nM 4OHT for 6 days. Representative images of nuclei stained with DAPI for the conditions indicated (scale bar = 10 μm). Percentage indicates the number of SAHF-positive cells within the population (see **d**). **c** Quantification of nuclear area, standard deviation of DAPI intensity (c) and the number of SAHF-positive cells (d) for the conditions indicated in b. Lines indicate the mean value of each replicate. n = 3 (c) and n = 4 (d) biologically independent replicates. Values of individual replicates for nuclear area and standard deviation are shown in Supplementary Fig. 1b, d. **e** Time series analysis of SAHF-positive nuclei following the addition of 100 nM 4OHT to IMR90 ER:HRASG12V cells in the presence or absence of ectopic dnMAML1 or 10 μM DAPT (left). n = 3 biologically independent replicates. Representative DAPI images of the indicated conditions (+RAS = 7 days of 4OHT treatment; scale bar = 25 μm). **c** Statistical significance calculated using one-way ANOVA with Tukey’s correction for multiple comparisons. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001
Fig. 2 NOTCH1 and JAG1 can non-autonomously repress SAHF formation in adjacent cells. a Schematic showing experimental set-up. IMR90 cells expressing doxycycline (DOX)-inducible N1ICD-FLAG were cultured with IMR90 ER:HRAS<sup>G12V</sup> cells expressing mRFP with 100 nM 4OHT ± 1000 ng/mL DOX for 6 days. b Quantification of SAHF-positive red cells for the experiment outlined in a. Alone: mono-cultured IMR90 ER:HRAS<sup>G12V</sup> cells; iN1ICD: DOX-inducible N1ICD-FLAG. c Representative images of co-cultures indicated (scale bar = 25 μm). Insets are unmerged DAPI images of the indicated cells (arrows). d Schematic showing experimental set-up. IMR90 ER:HRAS<sup>G12V</sup> cells expressing mRFP were co-cultured with RPE1 cells stably expressing either mVenus or JAG1-mVenus for 6 days ±100 nM 4OHT. e Quantification of SAHF-positive red cells for the experiment outlined in d. f Representative images of co-cultures indicated (scale bar = 25 μm). Insets are unmerged DAPI images of the indicated cells (arrows). Note DAPI foci in RPE1 cells are not SAHFs. b, f Lines indicate the mean value of individual replicates. n = 3 biologically independent replicates for all conditions. Statistical significance calculated using one-way ANOVA with Tukey’s correction for multiple comparisons; *p ≤ 0.001, NS = not significant.
Fig. 3 NOTCH1 signalling represses SAHF formation partially by repressing HMGA proteins. a–d qRT-PCR (n = 6) (a) and immunoblotting (b) for the indicated mRNA and proteins in IMR90 ER:HRASG12V cells stably infected with control vector or N1ICD-FLAG ± 100 nM 4OHT for 6 days. c, d qRT-PCR (n = 4) (c) and immunoblotting (d) of IMR90 cells expressing doxycycline (DOX)-inducible N1ICD-FLAG (N1ICD) and infected with a mVenus control vector or dnMAML1-mVenus ±1000 ng/mL DOX for 3 days. e Quantification of SAHF in IMR90 cells expressing ER:HRASG12V, N1ICD and EGFP or an EGFP-HMGA1 fusion ±100 nM 4OHT for 6 days and ±1000 ng/mL DOX for 3 days. f qRT-PCR for the indicated mRNA in IMR90 cells expressing rThy1-mRFP and co-cultured with RPE1 cells expressing mVenus or JAG1-mVenus, isolated using MACS (n = 3). Statistical significance calculated using one-way ANOVA with Tukey’s correction for multiple comparisons (a, c) or two-sample t-test (e, f). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

senescence than SAHFs (Supplementary Fig. 1a)13. However, in marked contrast to RIS, NIS cells lacked SAHFs (Fig. 1b, d).

To ask whether NIS cells simply lack SAHFs or whether N1ICD actively modulates chromatin structure, we expressed N1ICD in the presence of HRASG12V induced using 100 nM of 4OHT for 6 days. Interestingly, N1ICD in the context of RIS also resulted in a dramatic enlargement of nuclei but a complete ablation of SAHF formation (Fig. 1b–d). This was emphasised by a ‘smoothening’ of chromatin as indicated by a marked reduction in the standard deviation of 4,6-diamidino-2-phenylindole (DAPI) signal measured within individual nuclei (Fig. 1b, c; Supplementary Fig. 1b–d). We have previously shown that ectopic ablation of SAHF formation (Fig. 1b) resulted in a dramatic enlargement of nuclei but a complete downregulation at full senescence5. Thus our data indicate that NOTCH is dominant over RIS in terms of chromatin phenotype as well as SAMP composition.

In IMR90 ER:HRASG12V cells, RIS develops progressively over a time period of ~6 days following the addition of 4OHT50. NOTCH1 signalling is temporally regulated during RIS, where cleaved and active N1ICD is transiently upregulated before downregulation at full senescence5. To examine the temporal effects of NOTCH1 signalling on SAHF formation, we performed a time course experiment in IMR90 ER:HRASG12V cells. Cells were retrovirally infected with a dominant-negative form of MAML1 fused to mVenus (dnMAML1-mVenus) or treated with the γ-secretase inhibitor N-[3,5-difluorophenyl]acetyl]-l-allyl-2-phenyl[glycine-1,1-dimethylethyl ester (DAPT) to repress downstream signalling by N1ICD (Fig. 1a). We found that a greater number of SAHF-positive cells were formed and that these accumulated at earlier time points when NOTCH1 signalling was repressed (Fig. 1e). Furthermore, a dose-dependent effect was evident where higher concentrations of DAPT resulted in a greater proportion of cells developing SAHF than SAHFs (Supplementary Fig. 1g). Together, our data suggest that NOTCH1 activity can reverse SAHF after they have formed, we infected IMR90 cells with doxycycline (DOX)-inducible N1ICD-FLAG and constitutive HRASG12V. The addition of DOX after the establishment of senescence was sufficient to reduce the number of SAHF-positive cells and the standard deviation of DAPI signal, suggesting some degree of reversibility (Supplementary Fig. 1g).
**Fig. 4** Chromatin accessibility reflects gene transcription in RIS and NIS cells. **a** Diagram illustrating the method of ATAC-seq. **b** Genome browser images showing normalised ATAC-seq coverage and an active enhancer-associated histone modification (H3K27ac) in the cell conditions indicated around the GAPDH and IL1A genes. Unchanged = accessibility unaltered in RIS or NIS cells relative to growing; Opened in RIS = more accessible in RIS vs. growing cells; Opened in NIS = more accessible in NIS vs. growing cells (by edgeR and THOR). **c** Number of regions that become more accessible and less accessible in RIS and NIS cells relative to growing cells (intersect of edgeR and THOR). **d** Number of regions that are more accessible in RIS that are overlapping with a region that is more accessible in NIS (vs. growing). **e** Annotation of more (opened) and less (closed) accessible regions in RIS and NIS cells to genomic regions. **f** Accessible regions within the indicated subsets were annotated to genes if within 500 bp of a TSS. The average log$_2$-fold expression change (by mRNA-seq) of genes in RIS or NIS cells relative to growing cells is plotted. Values are mean ± s.d. Statistical significance calculated using a one-way ANOVA with Tukey's correction for multiple comparisons. ***p ≤ 0.001. **g** Gene ontology analysis (GO Biological Process 2015) using the TSS proximal accessible regions described in **f**.
signalling has a chromatin 'smoothening' effect that antagonises SAHF formation.

**Non-cell autonomous regulation of SAHFs.** N1ICD-expressing cells can induce NIS in adjacent normal cells, at least in the case of IMR90 fibroblasts. To determine whether N1ICD-expressing cells can also alter chromatin structure in adjacent cells, we performed co-cultures between mRFP1-expressing IMR90 ER:HRASG12V and IMR90 cells expressing DOX-inducible N1ICD-FLAG in the presence and absence of 4OHT and DOX (Fig. 2a). Strikingly, co-culture with N1ICD-expressing IMR90 cells was sufficient to repress SAHF formation in adjacent RPE (red) cells (Fig. 2b, c).

Of the canonical NOTCH1 ligands, we have previously observed a strong and unique upregulation of JAG1 following ectopic N1ICD expression, which we found to be responsible for the juxtafacic transmission of NIS. We reasoned that N1ICD-mediated upregulation of JAG1 and subsequent 'lateral induction' of NOTCH1 signalling is a likely mechanism by which SAHFs are regulated non-autonomously. To test this hypothesis, we expressed ectopic JAG1 fused to mVenus (JAG1-mVenus) in RPE1 JAG1-mVenus cells, but not control retinal pigment epithelial (RPE1) cells. We confirmed cell surface expression of ectopic JAG1 by flow cytometry (Supplementary Fig. 2a) before co-culturing with mRFP1-expressing IMR90 ER:HRASG12V cells. RPE1 JAG1-mVenus cells, but not control RPE1 cells, significantly repressed the formation of SAHFs (Fig. 2e, f). Note that this repression did not occur when these two types of cells were co-cultured without physical contact in a transwell format (Supplementary Fig. 2b). Our data suggest a mechanism by which lateral induction of NOTCH signalling by JAG1 can block SAHFs in the context of RIS, i.e. higher order chromatin structure can be regulated through cell–cell contact.

**NOTCH signalling represses the expression of HMGA genes.** To unravel the mechanisms underpinning NOTCH1-dependent repression of SAHFs, we re-analysed previously published RNA-seq data generated from IMR90 cells expressing HRASG12V and N1ICD. We found that N1ICD dramatically represses the expression of HMGA1 and HMGA2 (Supplementary Fig. 3a), critical components of SAHF structure.

To validate that NOTCH1 signalling represses HMGAs, we introduced constitutive N1ICD into IMR90 ER:HRASG12V cells. Ectopic N1ICD significantly repressed HMGA1 and HMGA2 at an mRNA and protein level in both the presence and absence of 4OHT-induced HRASG12V (Fig. 3a, b). The enforced expression of N1ICD after senescence establishment also resulted in the reduction of HMGA1 albeit to a lesser extent than pre-senescence N1ICD expression (Supplementary Fig. 1g). N1ICD has a similar effect on HMGA1 and 2 protein levels when expressed in other cell lines in the absence of HRASG12V, suggesting a conserved mechanism (Supplementary Fig. 3b). In the DOX-inducible N1ICD-FLAG system, inhibition of NOTCH1 signalling by co-expression of dnMAML1-mVen uses sufficient to rescue N1ICD-mediated repression of HMGA1 and HMGA2 by 4OHT (Fig. 3c, d), suggesting the effect is dependent on the canonical pathway of NOTCH signalling.

Finally, we used IMR90 ER:HRASG12V cells expressing DOX-inducible N1ICD-FLAG to investigate whether ectopic re-expression of EGFP-tagged HMGA1 is sufficient to rescue SAHFs. The introduction of EGFP-HMGA1 resulted in a partial, but significant, rescue of SAHF-positive cells when cells were treated with DOX and 4OHT (Fig. 3e).

Collectively, our data suggest that NOTCH1 signalling represses the formation of SAHFs at least partially by inhibiting HMGAs.

**Non-cell autonomous inhibition of HMGAs.** To determine whether HMGAs are repressed non-autonomously by JAG1 expressing cells, we performed further co-cultures between RPE1 cells retrovirally infected with JAG1-mVenus and IMR90 cells ectopically expressing a cell surface marker, rat-Thy1, allowing for subsequent isolation using magnetic-activated cell sorting (MACS) (Fig. 3f). As expected, IMR90 cells co-cultured with JAG1-expressing cells upregulated canonical NOTCH1 target genes, HEY1 and HEY2. Both HMGA1 and HMGA2 were significantly repressed in the same IMR90 cells (Fig. 3f), demonstrating that HMGA proteins can be repressed non-cell autonomously.

**Altered chromatin accessibility in RIS and NIS.** To investigate whether NOTCH1 influences chromatin structure at a higher resolution, we employed ATAC-seq (assay for transposase-accessible chromatin using sequencing)31. This method exploits a hyperactive Tn5 transposase that inserts sequencing adapters into regions of accessible chromatin. Following adapter-primed PCR amplification, these regions were sequenced to identify accessible regions of chromatin genome wide (Fig. 4a).

We generated at least three replicates from IMR90 ER:HRASG12V cells expressing N1ICD-FLAG or a control vector and induced with 4OHT or not. For simplicity, these conditions were labelled as ‘Growing’, ‘RIS’, ‘NIS’ and ‘N+RIS’ (expressing both N1ICD and RAS). Using a previously published normalisation approach,32 we generated normalised coverage files that appeared comparable to each other, especially around housekeeping genes (Fig. 4b). Most of the samples, excluding a single replicate from the NIS and N+RIS conditions (which were excluded from downstream analysis), were of high quality with a ‘reads in peaks’ percentage (RiP%) of >10% (Supplementary Fig. 4a). Replicates clustered well by unbiased principal component analysis (PCA) (Supplementary Fig. 4b). Moreover, our samples clustered with publically available ATAC-seq and DNase-seq data generated from IMR90 cells (Supplementary Fig. 4c), but separated from other cell types (BJ, HaCaT, MCF710A and HEKn cells).

Using MACS peak calling, we found that the number of peaks identified in each replicate of a condition was similar and that, in general, chromatin accessibility was dramatically increased in RIS (145,649 consensus peaks detected in ≥2 replicates) and NIS cells (149,877 peaks) relative to growing cells (83,920 peaks) (Supplementary Fig. 5a). To quantitatively identify regions of altered accessibility in RIS and NIS cells relative to growing cells, we performed differential binding analysis using both edgeR33,34 and THOR35 before taking only regions identified by both methods for downstream analysis (Supplementary Data 1). Using this stringent approach, we identified 44,556 regions that become significantly more accessible (opened) and 9603 regions that become significantly less accessible (closed) in RIS cells relative to growing cells. In NIS cells, 20,499 regions became more accessible and 15,444 regions less accessible (Fig. 4c). Despite the robust gain of chromatin accessibility in both types of senescence, there were relatively few shared sites (Fig. 4d).

A previous study mapping chromatin accessibility in replicatively senescent cells using FAIRE-Seq found that gene-distal regions, especially repeat regions, become relatively more open whereas genic regions become closed compared to growing fibroblasts. Consistently, regions of increased accessibility in RIS and NIS cells were enriched at gene-distal sites
Fig. 5 Ectopic N1ICD and HMGA1 knockdown antagonise chromatin opening in RIS. **a** Unbiased clustering of replicates for the conditions indicated. **b**, **c** Volcano plots showing regions of altered accessibility in N+RIS cells (b) and RIS+shHMGA1 (c) cells relative to RIS cells. Regions that are also opened in RIS (red) and NIS (purple) relative to growing cells are indicated. **d** Number of novel accessible regions in RIS (identified in Fig. 4c) that are repressed by N1ICD (significantly reduced in N+RIS vs. RIS) and are HMGA1 dependent (significantly reduced in RIS+shHMGA1 vs. RIS). The Venn diagram shows the number of regions repressed by N1ICD that are overlapped by a region dependent on HMGA1. **e** Genomic GC percentage of the accessible regions indicated. **f** K-means clustered heatmap showing the enrichment of normalised reads around accessible regions altered in any of the conditions relative to growing cells. **g** Genomic GC percentage of the clusters indicated, identified in **f**, **e**, **g** Mean ± s.d is plotted. Statistical significance calculated using one-way ANOVA with Tukey’s correction for multiple comparisons; ***p ≤ 0.001
with the majority of opened regions mapping to enhancer, intergenic, intronic and repeat regions (Fig. 4e). Many of these repeat regions were further annotated as long interspersed elements, long-terminal repeats, short interspersed elements and simple repeat regions (Supplementary Fig. 5c, d), although these values may be underestimated due to the exclusion of multi-mapping reads from our data. Many of the regions that became less accessible in RIS cells relative to growing cells were closer to transcriptional start sites (TSSs) (Supplementary Fig. 5b) and mapped to exons, CpG-islands and untranslated regions (UTRs) (Fig. 4e). In contrast to replicative senescence, regions of decreased accessibility in NIS cells mostly mapped to
C/EBP revealed that regions opened in RIS were highly enriched for the important role of C/EBP of more accessible regions in RIS (identi number of significant alterations (log2 fold change < -0.58 or >0.58 and FDR < 0.01) and <<-1 indicates the number with a log2 fold change of <<-1. I Number of more accessible regions in RIS (identified in Fig. 4c) that are repressed by co-culture with MCF7, A549 and Hep3B. Statistical significance calculated using one-way ANOVA with Tukey's correction for multiple comparisons; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, NS = not significant.

Altered accessibility of genes reflects expression. Chromatin accessibility at regulatory elements has been correlated with gene expression. To determine whether genetic alterations to chromatin accessibility in RIS and NIS reflects gene expression, we assigned regions of altered accessibility (opened or closed in RIS or NIS) to genes if within 500 bp of a TSS (Fig. 4f). On average, genes that were opened in RIS relative to growing cells were also transcriptionally upregulated by mRNA-seq in RIS relative to growing cells (Fig. 4f, top). Genes that were opened in NIS cells were transcriptionally upregulated in NIS cells, while less accessible genes were transcriptionally repressed (Fig. 4f, bottom). Consistent with our previous RNA-seq data, genes that became more accessible in RIS were significantly enriched within gene ontology (GO) terms such as 'inflammatory response' and 'cytokine secretion', reflecting the inflammatory secretome produced by RIS cells (Fig. 4g). Genes that became less accessible in RIS were enriched within GO terms such as 'regulation of cell cycle' (Fig. 4g), perhaps reflecting non-proliferative features of RIS (although average gene expression of this gene set was not significantly altered). Unbiased motif enrichment analysis revealed that regions opened in RIS were highly enriched for the C/EBPβ-binding motif (Supplementary Fig. 5e), consistent with the important role of C/EBPβ in regulating the inflammatory SASP. Regions opened in NIS were enriched with the RBP-J-binding motif (Supplementary Fig. 5f), a critical DNA-binding factor downstream of NOTCH signalling. Normalised ATAC-seq coverage files, when viewed using a genome browser (Fig. 4b, Supplementary Fig. 6a-d), demonstrated increased accessibility around transcriptionally activated genes. We also noted that, while the accessibility at many promoters was unaltered in RIS cells, some transcriptionally activated genes, such as IL1A and HMGA1, were proximal to enhancer elements that became more accessible (Fig. 4b, Supplementary Fig. 6a). Together, these data demonstrate that RIS and NIS cells have unique open chromatin landscapes and that (gene proximal) alterations reflect their transcriptional landscapes.

NOTCH signalling antagonises chromatin opening in RIS. By unbiased clustering of ATAC-seq data, we observed a greater correlation between NIS and N+RIS cells than between RIS and N+RIS cells (Fig. 5a). This suggests a dominant effect of N1ICD over RAS on the nucleosome scale, consistent with our previous observations for SASP components and SAHFs (Fig. 1d).

To determine whether NOTCH1 signalling can repress the chromatin alterations observed in RIS in favour of a 'NIS-like' chromatin landscape, we focussed on the 44,556 regions that became significantly more accessible in RIS cells relative to growing cells (referred to as 'RIS-driven accessible regions', Fig. 4c) and the 20,499 regions that became significantly more accessible in NIS cells relative to growing cells (referred to as 'NIS-driven accessible regions', Fig. 4c). By comparing chromatin accessibility of N+RIS cells with RIS cells we found that formation of many RIS-driven accessible regions (62.7%) were repressed by N1ICD expression (Fig. 5b). N1ICD expression also increased the accessibility of NIS-driven accessible regions (Fig. 5b). When viewed in the genome browser, it was evident that N1ICD expression can repress the formation of accessible regions located at enhancer elements upstream of the HMGA1 promoter in RIS cells (Supplementary Fig. 6a), although we failed to detect any alterations at the HMGA2 locus (Supplementary Fig. 6b), providing a potential mechanism for NOTCH1-mediated repression of HMGA1.

HMGA proteins have previously been shown to affect chromatin compaction. To determine whether repression of HMGA1 is a mechanism by which N1ICD can repress formation of RIS-driven accessible regions, we generated additional ATAC-seq samples from IMR90 ER:HRAS<sup>G12V</sup> cells expressing a short hairpin against HMGA1 and treated with 4OHT, hereafter referred to as 'RIS+shHMGA1'. By comparing RIS+shHMGA1 with RIS, we identified 8909 RIS-driven accessible regions that were dependent on HMGA1 (Fig. 5c). Of these, 69.9% (6168) were also repressed by N1ICD (Fig. 5d). These analyses illustrate that a subset of RIS-driven accessible regions can be repressed by N1ICD, possibly by HMGA downregulation. However, HMGA1 knockdown was not sufficient to induce the formation of NIS-driven accessible regions (Fig. 5c), suggesting an HMGA1-independent mechanism in the formation of these sites. RIS-driven accessible regions (opened in RIS) were significantly more AT-rich than NIS-driven accessible regions (opened in NIS) or regions with reduced accessibility (Fig. 5e), supporting the involvement of HMGA1 in the formation of RIS-driven accessible regions.

To validate the above approach, we used our normalised coverage files to perform unbiased k-means clustering centred around accessible regions that were altered in either RIS or NIS cells (opened or closed relative to growing cells) (Fig. 5f). Accessible regions separated into clusters that were dominated by either the RIS (clusters 1 and 2) or NIS (clusters 3 and 4) conditions. Strikingly, the signal in RIS-dominated clusters, cluster 2 in particular, was reduced in the N+RIS and RIS+shHMGA1 conditions when compared to the RIS condition (Fig. 5f). Consistently, cluster 2 was more AT-rich than cluster 1 (Fig. 5g), supporting a role for HMGA1. Notably, while peaks in clusters 3 and 4 were increased in the N+RIS condition, they did not increase in the RIS+shHMGA1 condition, reinforcing an HMGA1-independent mechanism of chromatin opening in NIS (Fig. 5f). Therefore, in line with microscopic SAHF structures, N1ICD alters chromatin structure in RIS at the nucleosome scale in part by repressing HMGA1 expression.
Non-cell autonomous regulation of SAHFs by tumour cells. Both HMGA1 and NOTCH1 can act as oncogenes or tumour suppressors in a context-dependent manner. We reasoned that the relationship between these two genes might also be important in the tumour microenvironment and asked whether tumour cells expressing JAG1 can affect HMGA1 expression and chromatin structure in adjacent fibroblasts.

To answer this question, we used the Cancer Cell Line Encyclopedia\textsuperscript{36} to identify tumour cell lines that express low (MCF7), medium (A549) and high (Hep3B) levels of JAG1, which we confirmed by immunoblotting (Fig. 6a). Co-culture of tumour cell lines with IMR90 cells expressing both ER:HRAS\textsuperscript{G12V} and NOTCH1 (Fig. 6b, c). Consistent with our previous experiments (Fig. 1e, Supplementary Fig. 1e), the canonical NOTCH pathway was completely abrogated by DAPT, suggesting the effect is dependent on the SAHF formation in red (RIS) cells in a contact-dependent manner.

To determine whether tumour cell lines can induce NOTCH1 signalling and repress HMGAs non-autonomously, we repeated the co-cultures and isolated the IMR90 ER:HRAS\textsuperscript{G12V} mRFP1 cells using flow cytometry (Fig. 6d, Supplementary Fig. 7a). The number of SAHF-positive IMR90 cells above basal levels both in monoculture (Fig. 6d) and co-culture (Fig. 6b).

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In contrast to control or parental A549 cells, co-culture of JAG1-knockout A549 cells with red IMR90 ER:HRAS<sup>G12V</sup> cells in the presence of 4OHT had little effect on SAHF formation in red (RIS) cells (Fig. 6g).

In addition to JAG1-knockout A549 cells, we generated MCF7 cells containing DOX-inducible JAG1 fused to mVenus (JAG1-mVenus). By immunoblotting, we observed low-level expression of JAG1-mVenus even in the absence of DOX, likely caused by ‘leaky’ transcription (Supplementary Fig. 7g). Addition of 10 ng/ mL of DOX to the culture was sufficient to induce JAG1 to comparable levels as those observed endogenously in Hep3B cells (Supplementary Fig. 7g). Co-culture of MCF7 cells containing inducible JAG1 with red IMR90 ER:HRAS<sup>G12V</sup> cells was sufficient to reduce the number of SAHF-positive red cells even in the absence of DOX (reflecting the slightly increased levels of JAG1) and completely repress SAHF formation in red cells in the presence of DOX (Supplementary Fig. 7h). While we cannot exclude the effects of other cell-contact-mediated signalling pathways on chromatin structure, our data together demonstrate that JAG1-expressing tumour cells can repress SAHF formation in adjacent senescent cells in a JAG1-dependent manner.

Non-cell autonomous regulation of chromatin accessibility. Next, we asked whether tumour cell lines could repress the formation of RIS-driven accessible regions in fibroblasts, as was the case for ectopic N1ICD (Fig. 5b). Utilising flow cytometry, we isolated 4OHT-induced IMR90 ER:HRAS<sup>G12V</sup> mRFP1 cells after co-culture with tumour cell lines and performed ATAC-seq (Fig. 6d). We found that 66% (29,507), 72% (32,364) and 75% (33,581) of RIS-driven accessible regions were significantly repressed by co-culture with MCF7, A549 and Hep3B cells, respectively (Fig. 6h; Supplementary Fig. 8a, b). Co-culture with MCF7, A549 or Hep3B cells induced opening of 6948, 10,303 and 14,064 NIS-driven accessible regions, respectively (Fig. 6h). These data correlated well with the ability of the tumour cell lines to repress SAHFs in adjacent IMR90 (Fig. 6b) and the JAG1 levels expressed by each line (Fig. 6a). RIS-driven accessible regions repressed by co-culture with tumour cell lines overlapped well with each other and with regions repressed by ectopic N1ICD (Supplementary Fig. 8c, d). These data suggest that tumour cells expressing JAG1 can dramatically alter the chromatin landscape of adjacent stromal cells at the nucleosome level.

**HEYL and HMGA1 anti-correlate in multiple tumour types.** If NOTCH1 signalling inhibits HMGA1 in vivo, we would expect an anti-correlation between NOTCH1 activity and HMGA1 expression in human tumour samples. To test this, we first performed a pan-tissue-type analysis using expression microarray data from the R2 database (http://r2.amc.nl) by comparing the expression of HMGA1 and canonical NOTCH1 target genes. When Z-score expression values were analysed in 36,846 human samples, we observed a significant negative correlation between HMGA1 and HEYL (R = −0.356, p < 0.0001) and HMGA1 and HEY1 (R = −0.281, p < 0.0001), but no correlation between HMGA1 and HES1 (Supplementary Fig. 9a, b, c). Interestingly, HEYL and HEY1, but not HES1, were also significantly upregulated in IMR90 fibroblasts co-cultured with JAG1-expressing RPE1 cells (Fig. 3g). To study the prognostic importance of this relationship, we used the web-based tool KM-plotter<sup>39,40</sup> and found that patients with low HMGA1 or high HEYL have a significantly better prognosis in lung adenocarcinoma, but not in lung squamous cell carcinoma (SCC) (Supplementary Fig. 9d, e), suggesting that the relationship between these proteins may have prognostic value in certain types of cancer. High HEYL levels were prognostic of better overall survival in both types of lung cancer patient (Supplementary Fig. 9d, e).

As microarray data can be dependent on the quality of the probe used, we analysed the co-expression of HMGA1 and HEYL or HEY1 using RNA-seq data generated by The Cancer Genome Atlas (TCGA) Research Network<sup>41</sup> (http://cancergenome.nih.gov). There was a significant negative correlation between HMGA1 and HEYL in the majority of tumour types analysed (Fig. 7a) and a particularly strong anti-correlation in lung SCC (Fig. 7b) (R = −0.4842; p < 0.0001). When TCGA patients with lung SCC were categorised based on expression into ‘HMGA1 high–HEY1 low’ and ‘HMGA1 low–HEY1 high’ tumours, patients in the former category had a better overall survival (Fig. 7c) (p = 0.00316). We also found a significant negative correlation between HMGA1 and HEY1 in various cancer types, which were not completely overlapping with those where HMGA1 and HEYL anti-correlate (Supplementary Fig. 10a). For example, they were not negatively correlated in lung SCC and the expression of these two genes was not prognostic of patient survival (Supplementary Fig. 10b, c). However, kidney renal clear cell carcinoma showed the strongest negative correlation between HMGA1 and HEY1 and their expression patterns were indicative of prognosis (Supplementary Fig. 10a, d, e). Together, these data demonstrate that an anti-correlation between HMGA1 expression and NOTCH1 activity is evident in cancer and that this correlation can be prognostic of patient outcome.

**Discussion**

In the current study, we provide evidence for NOTCH-mediated ‘lateral modulation’ of chromatin structure at the microscopic and nucleosome scales. While RIS cells form prominent SAHFs at the microscopic scale,<sup>13,42</sup> at the nucleosome scale we observed a robust increase in chromatin accessibility. Both SAHFs and RIS-
driven accessibility can be inhibited by N1ICD-mediated repression of HMGA1 (Fig. 8). While the essential and structural role for HMGA1 in SAHF formation is well established, its role in chromatin accessibility is unclear. HMGA proteins compete with Histone-H1 for linker DNA and thus affect chromatin compaction, as demonstrated by techniques such as fluorescence recovery after photo bleaching and MNase digestion assays14,15. Our data, using sequencing technology, demonstrate that HMGA1 effects the formation of ectopic accessible regions, potentially by facilitating the binding of other transcription factors, such as C/EBPβ, identified here through motif analysis of RIS-driven accessible regions. It is known that chromatin accessibility is an indicator of developmental maturity15 and that cancer cells acquire ectopic accessible regions54,56. For example, during the metastasis of small cell lung cancer, a dramatic increase in chromatin accessibility at distal regulatory elements allows tumour cells to co-opt pre-programmed gene expression programmes, providing a growth advantage56. Thus our data raise a possibility that HMGA1 can drive pluripotency and cancer in part by modulating chromatin accessibility. It will be important to understand how HMGA1 facilitates both chromatin ‘opening’ at the nucleosome scale and the formation of SAHFs and to determine whether the two are related. We wonder whether the subset of HMGA1-dependent regions that are gene distal could have structural rather than regulatory functionality.

Chromatin accessibility was also increased in NIS cells although these were often at distinct loci. Unlike RIS cells, NIS cells do not form SAHFs and are instead characterised by chromatin ‘smoothening’. The mechanisms of chromatin smoothening and formation of RIS-driven accessible regions, and whether these events are related, remains unclear. Note, although knockdown of HMGA1 blocked formation of SAHFs and many RIS-driven accessible regions, it was not sufficient to induce NIS-like chromatin smoothening or NIS-like chromatin accessibility, thus NOTCH signalling modulates chromatin by both HMGA1-dependent and -independent mechanisms (Fig. 5b, c). One possible mechanism through which NOTCH modulates chromatin is through directed histone acetylation47–50. N1ICD activates gene transcription by recruiting histone acetyltransferases11 and was more recently shown to drive rapid and widespread deposition of H3K9ac51, which is known to be associated with nucleosome assembly, particularly in DNA replication and repair52. NOTCH signalling can be transiently activated during stress-induced senescence (e.g. oncogene- and DNA damage-induced senescence)9 but also plays important roles during development and in cancer, thus ‘lateral induction’ of NOTCH activity through JAG1 could affect chromatin structure in various biologically relevant scenarios involving epithelial and/or fibroblast cells. Here we extend our analysis to the more specific ‘epithelial-fibroblast’ scenario that might mirror the cancer microenvironment where epithelial tumour cells are in active communication with stromal cells through the NOTCH1–JAG1–HMGA1 signalling axis. Consistently, using the Pten-null mouse model of prostate cancer, Su and colleagues53 demonstrated that JAG1 expression in tumour cells facilitates the formation of a ‘reactive stroma’, which plays an important role in tumour development. It will be important to test whether chromatin structure is altered in the stroma of such tumours and whether this is dependent on HMGA1 repression. In NOTCH-ligand-expressing tumours, targeting chromatin-modifying enzymes in the stromal compartment may present a unique therapeutic opportunity to alter the tumour niche.

**Methods**

**Cell culture.** IMR90 HDFs (ATCC) were cultured in Dulbecco’s modified Eagle’s medium (DMEM)/10% foetal calf serum (FCS) in a 5% O2/5% CO2 atmosphere. hTERT-RPE1 cells (ATCC) were grown in DMEM-F12/10% FCS in a 5% O2/5% CO2 atmosphere. MCF7, H1299, A549 and Hep3B cells (ATCC) were grown in DMEM/F12 medium supplemented with 10% heat inactivated FCS. Cell identity was confirmed by STR (short tandem repeats) genotyping. Cells were regularly tested for mycoplasma contamination and always found to be negative.

Co-cultures were set-up at a cell number ratio of 1:1 and performed in DMEM/ 10% FCS in a 5% O2/5% CO2 atmosphere. For transwell experiments, IMR90 cells were plated in the bottom chamber and UTTER-PRE1 or tumour cells were plated into the top chamber of a Corning 12-well Transwell plate (CLS3460 Sigma). The following compounds were used in cultures: 100 nM 4-hydroxymatuximab (40HT) (Sigma), 10 µM DAPT (Sigma), 100 µM etoposide (Sigma), between 10 and 1000 ng/ml doxycycline (DOX) (Sigma) as indicated in individual figures.

**Vectors.** The following retroviral vectors were used: pLNCX (dolchent) ER: HRAG12LW, pPWZl-hyrgo for N1ICD–FLAG (residues 1758–2556 of human NOTCH1) and mRFP1; pLPC-puro for dnMAML1-mRFP (residues 12–74 of human MAML1); mRFP1, rThy1-mRFP1, JAGG1D1-mRFP1 and mVenus; pQCXII-H for DOX-inducible NICD–FLAG2; MSCV-puro for mi30 shHMGA1 (shHMGA1 target sequence 5'-ATGAGACAAATGCTGATGTAT-3'); and pCLIP24 for pCLIP1 JAGG1D1-mRFP1. To generate pLPC-puro rThy1-mRFP1, we first PCR cloned mRFP into pLPC-puro (pLPC-puro-x-mRFP, where x denotes cloning sites to express mRFP-fusion proteins). The CDS of rat-Thy1 was PCR amplified from cDNA (a gift from M. de Rocquigny, CHUK Institute, UK), removing the stop codon, before cloning into pLPC-puro-x-mRFP. To generate pLPC-JAGG1-D1-mRFP1, the CDS of human JAGG1 was amplified using cDNA derived from N1ICD-expressing IMR90 cells, removing the stop codon, before cloning into pLPC-puro-x-mRFP. To generate pCLIP1 (DOX-inducible) JAGG1D1-mRFP1, JAGG1D1-mRFP1 was subcloned using PCR into pCLIP1.

**Flow cytometry.** Analysis of ectopic JAG1-mRFP expression was conducted by flow cytometry5. Cells were fixed using 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) and stained with anti-JAG1-FLAG (FAB1726A, R&D Systems, 1:10) or isotype control antibody (IC0041A, R&D Systems, 1:10) before analysis on a FACScalibur flow cytometer (Becton Dickenson). Flow data were further analysed using FlowJo v10.

**MACS and FACS.** MACS of rThy1-expressing cells was performed using CD90.1 microbeads (130-094-523, Miltenyl Biotec) according to the manufacturer’s instructions. Fluorescence-activated cell sorting (FACS) using an Influx (Becton Dickenson) flow cytometer.

**Fluorescence microscopy.** Analysis was performed as previously described16. Briefly, cells were plated onto 1.5 glass coverslips the day before fixation to achieve approximately 60% confluence. Cells were fixed in 4% (v/v) PFA and permeabilised with 0.2% (v/v) Triton X-100 in PBS with DAPI. Coverslips were mounted onto Superfrost Plus slides (4951, Thermofisher) with Vectashield Antifade mounting medium (H-1000, Vector Laboratories Ltd.). Images were obtained using a Leica TCS SP8 microscope with a HC PL APO CS 1.4NA 100x oil objective (Leica Microsystems). At least 30 nuclei were captured per biological replicate and condition before Fiji was used to calculate nuclear area, standard deviation and maximum intensity of DAPI signal per nucleus. Specifically, the DAPI channel was duplicated, desaturated and a threshold applied using the 0tsu method before holes were filled and the ‘analyse particles’ function was used to create a region of interest per nucleus for measurement in the original DAPI-stained image. SADS were visualised by DNA fibre formation in situ hybridisation as previously described15 using fluorescent probes that target the α-satellite repeat sequence (5'-CTT7TTGATAGAAGAGTTAGTTTAGACATCTTTGAGT-3'), the percentage of SAHFs- and SADS-positive cells was counted by scoring at least 200 cells per replicate and condition.

**Quantitative reverse transcription-PCR.** RNA was prepared using the Qiagen RNeasy Plus Kit (74136, Qiagen) according to the manufacturer’s instructions and reverse-transcribed to cDNA using the Applied Biosystems High-Capacity Reverse Transcription Kit (4388-683, Thermo Fisher). Relative expression was calculated as previously described10 on an Applied Biosystems Quantstudio 6 by the 2ΔΔCt method10 using β-actin (ACTB) as an internal control. The following primers were used:

- **ACTB forward:** 5’-GGACCTTGCGAAGAGAGAGTAGG-3’
- **ACTB reverse:** 5’-AGGAGAAGGGCTGGAAAGAGG-3’
- **HEY1 forward:** 5’-CTTCAATAATTGTTGATGC3’
- **HEY1 reverse:** 5’-CAAGCCTAAATCAGGAAGTTCC-3’
- **HEY1 forward:** 5’-CCGCTGTAGGTTGCTCATTGGG-3’
- **HEY1 reverse:** 5’-TCTTTGTTGGGCGGCGTGGG-3’
- **HES1 forward:** 5’-AGTGTCGAGGCGGCATTAATACG-3’
- **HES1 reverse:** 5’-ATGATGGCTGCTGATGCGATGG-3’
- **HMGA1 forward:** 5’-TAGGCTTACGACGGCAGAGGAA-3’
- **HMGA1 reverse:** 5’-TGGTTTCTCTTCGGCATTGGG-3’

The percentage of SAHF- and SADS-positive cells was counted by scoring at least 200 cells per replicate and condition.
**Protein quantification by immuno blotting.** Immuno blotting was performed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis gels using the following antibodies: anti-β-actin (Sigma, A5441, 1:10,000); anti-HRAS (Calbiochem, OP-23, 1:500); anti-NOTCH1 (Cell Signaling, 4380, 1:500); anti-HESI (Cell Signaling, 11998, 1:1000); anti-FLAG (Cell Signaling, 2368, 1:1000), anti-HMG1A (Cold Spring Harbor Labs, #37, 1:1000); anti-HMG1A (Abcam, Ab4078, 1:1000); anti-H3K27ac (Cold Spring Harbor Labs, #42, 1:1000); anti-GFP (Clontech 632377, 1:1000); and anti-JAG1 (Cell Signaling, 2155, 1:1000). Images of uncropped immunoblots are included in Supplementary Fig. 11.

**ATAC-seq.** ATAC-seq samples were generated as previously described using 100,000 IMR90 cells and 13 cycles of PCR amplification. Samples were size selected between 170 and 400 bp (in order to isolate ‘nucleosome free’ and ‘mono-nucleosome fragments) using SPRIselect beads (B23319, Beckman Coulter) before single-end sequencing to generate 75 bp reads on the NextSeq-500 platform (Illumina).

**HiP-seq.** Chromatin immunoprecipitation (ChIP) was performed as previously described using 20 µg of sonicated chromatin from growing and RIS IMR90 ER: HRASΔ1/G1V cells expressing a short-hairpin targeting the 5′ UTR of human HMG1A (RIS+shHMG1A). RNA was purified as above and quality checked using the Bioanalyzer eukaryotic total RNA nano series II chip (Agilent). mRNA-seq libraries were prepared from six biological replicates of each condition using the TrueSeq Stranded mRNA Library Prep Kit (Illumina) according to the manufacturer’s instructions except that size selection was performed after PCR amplification using SPRIselect beads (B23319, Beckman Coulter). Samples were sequenced single-end using 50 bp reads on the HiSeq-2500 platform (Illumina).

**RNA-seq.** RNA-seq data was generated from IMR90 ER:HRASΔ1/G1V cells expressing a short-hairpin targeting the 5′ UTR of human HMG1A (RIS+shHMG1A). RNA was purified as above and quality checked using the Bioanalyzer eukaryotic total RNA nano series II chip (Agilent). mRNA-seq libraries were prepared from six biological replicates of each condition using the TrueSeq Stranded mRNA Library Prep Kit (Illumina) according to the manufacturer’s instructions and sequenced using the HiSeq-2500 platform (Illumina).

**Generation of genome-edited JAG1 knockout clones.** The following CRISPR guides were designed against exon 2 of JAG1 (NM_002412.4) (Supplementary Fig. 7d):

- sgJAG1_2.1: 5′-AGTCGGCCGTACGCGGCCGGG-3′ (PM6-GGGG) and sgJAG1_2.2: 5′-CCGGGACGTACCTTCTTG-3′ (PM13-AGAG).
- Oligonucleotides (Sigma Aldrich) were cloned into pSpCas9(BB)-2A-GFP (p5).
- sgCas9(9B): 5′-AAGGTACGG-3′ and sgCas9(2B): 5′-ATAATCCG-3′.
- Oligos were cloned into pSpCas9(BB)-2A-GFP (PX548) as a gift from Feng Zhang (Addgene plasmid # 48138). Guide cutting efficiency was determined in A549 cells using the T7 assay (New England Biolabs, following manufacturer’s instructions). To generate independent, non-sister-clonal cell lines, A549 cells were transiently transfected (Lipofectamine 3000, Thermo Fisher Scientific) with PX548-empty, PX548-sgJAG1_2.1 and PX548-sgJAG1_2.2, and single cell clones were selected with 2 µg/ml of puromycin.

**ChIP-seq and ATAC-seq analysis.** THOR35 and edgeR33 were used to identify differentially accessible regions between conditions. For the comparisons ‘RIS vs. growing’ and ‘RIS vs. growing’, the intersect of regions detected by THOR and edgeR was taken. This approach gave us a robust set of regions that are altered in RIS and NIS conditions for further analysis. For comparisons where volcano plots were generated, edgeR was used to interrogate how different genetic and cell-culture manipulations effect the alterations detected in RIS.

**Differential accessibility analysis.** THOR35 and edgeR33 were used to identify differentially accessible regions between conditions. For the comparisons ‘RIS vs. growing’ and ‘RIS vs. growing’, the intersect of regions detected by THOR and edgeR was taken. This approach gave us a robust set of regions that are altered in RIS and NIS conditions for further analysis. For comparisons where volcano plots were generated, edgeR was used to interrogate how different genetic and cell-culture manipulations effect the alterations detected in RIS.

**Antibodies.** Anti-HMGA2 (Cold Spring Harbor Labs, #24, 1:1000); anti-GFP (Clontech 632377, 1:1000); anti-FLAG (Cell Signaling, 2368, 1:1000), anti-HMGA1 (Abcam, Ab4078, 1:1000); anti-H3K27ac (Cold Spring Harbor Labs, #42, 1:1000); anti-GFP (Clontech 632377, 1:1000); and anti-JAG1 (Cell Signaling, 2155, 1:1000). Images of uncropped immunoblots are included in Supplementary Fig. 11.
PCA analysis and correlation heatmaps. Samples were normalised with the pre-calculated normalisation factors (as described above in ‘Generation of normalised coverage files’), and reads from all growing, RIS, NIS, N+RIS, shHMGA1 and RIS/shHMGA1 consensus peak sets (present in at least two replicates across all of the samples) were extracted and used in the PCA analysis and the correlation analysis of data sets. Pearson correlation was calculated between samples based on these normalised read counts and correlation heatmaps were generated with pheatmap (http://CRAN.R-project.org/package=pheatmap) and WPGMA clustering. PCA plots were generated using ggplot273.

Reads from publicly available ATAC-seq and DNase-seq data sets74–77 (references and NIH Epigenomics Roadmap Initiative) were extracted from the same regions; however, since these were not included in normalisation factor calculation, standard CPM normalisation was used for Supplementary Fig. 4c.

Volcano plots. edgeR calculated statistical parameters (logFC and logFDR) were used to visualise differentially accessible regions in RIS (red) and NIS (purple) compared to growing cells in the comparisons indicated. Plots were generated using ggplot272.

Motif enrichment analysis. Meme-ChIP suite (version 4.12.0), together with Hocomore (version 11) human and mouse PWMs, was used to detect motif enrichment in a 600 bp region centred at the peak summit.

TCGA analysis. We analysed the expression levels of NOTCH-associated genes in the publicly available RNA sequencing data generated by the TCGA Research Network: http://cancergenome.nih.gov/41. Computational analysis and statistical testing of the Next-Generation Sequencing data was conducted using the 32 R statistical programming language.79 Filtered and log-normalised RNA expression data along with all available clinical data were downloaded from the GDAC firehose database (run: stddata_2015_06_01) for each gene of interest from the relevant cancer-specific collections.

Statistics and reproducibility. No statistical method was used to predict determinate sample size and experiments were not randomised. Statistical analyses were conducted using the Graphpad Prism 6 and R statistical software, except for TCGA data analysis (which was as described in the methods above). One-way analysis of variance with Tukey’s correction for multiple comparisons was used for data sets with >2 conditions. Two-sample t-tests were used for two-condition comparisons. The statistical tests were justified as appropriate based on the number of samples compared and the assumed variance within populations. A p-value of <0.05 was used to indicate statistical significance.

Data availability. The RNA-seq, ChIP-seq and ATAC-seq data generated for this study have been deposited at the Gene Expression Omnibus (GEO) with the accession number GSE103590. Gene expression data from RIS, NIS and N+RIS is previously published8 and available under GEO accession number GSE72404.

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Author contributions
A.J.P., M.H. and R.H.-H. designed experiments, performed experiments and analysed data. D.B., S.A.S. and A.J.P. analysed sequencing data. S.S. analysed TCGA data. E.M., A. S., P.D’S and I.A.R. generated A549 JAG1 knockout cell lines. H.K. provided the antibodies for H3K27ac and H3K4me1 ChIP-seq. S.R., S.A.S., H.K. and M.N. supervised experiments and interpreted the data. M.N., M.H. and A.J.P. conceived the project. M.N. and A.J.P. wrote the manuscript with contribution and review from all authors.

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