



DDR1 and Notch: a multifaceted synergy

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In recent years our appreciation of the function and design of many signalling pathways implicated in cancer has changed substantially. The original idea of intrinsically independent and linear routes has progressively evolved to the concept of complex networks, involving extensive crosstalk and interplay between various signalling pathways. Yet, we are only beginning to understand how these networks are interconnected within cancer cells. This knowledge will be essential for the design of effective drug combinations as well as for the anticipation of potential mechanisms of resistance.

This burden of complexity was evident in our recent manuscript reporting an efficacious preclinical therapy based on the combined pharmacological inhibition of the receptor tyrosine kinase DDR1 and Notch signalling in K-Ras driven lung adenocarcinoma (1). Yet, as pointed out in the recent commentary by Dr. Lendahl, we are still far from understanding the underlying biology and the hierarchy that governs this K-Ras/DDR1/Notch interplay. Indeed, both positive and negative interactions between Notch and Ras have been identified in various tumour types where Notch can sustain or prevent tumour growth depending on the cellular context (2). We hypothesize that in K-Ras driven lung adenocarcinoma this context dependency may in part rely on the function of DDR1.

We identified DDR1 as the top hit in the transcriptional profiling of a subset of mouse lung hyperplasias when analysed shortly after the activation of a resident K-Ras^{G12V} oncogene. The limited consensus on a reliable Notch-dependent transcriptional profile in the published literature prevented us from analysing the presence of a Notch signature in these early lesions. Yet, in the early stages of

pancreatic adenocarcinoma, oncogenic K-Ras fails to induce Notch target genes suggesting that these pathways converge subsequently during tumour initiation (3). Although we currently lack supporting experimental evidence, we can speculate on several non-mutually exclusive mechanisms that could explain this functional convergence.

In breast cancer cells, the interaction with DDR1 triggers Notch activation (4). We are still ignorant as to whether the same process operates in K-Ras-driven lung adenocarcinoma. If this is the case, and since DDR1 transcription is partly controlled by MAPK activity (5), K-Ras-induced DDR1 expression would precede Notch activation. Yet, it is currently unclear how DDR1 itself becomes activated. Collagens, the only known DDR1 ligands (5) are among the most abundant proteins in mammalian tissue suggesting that additional layers of regulation must exist to prevent unscheduled activation. RTKs are not uniformly activated across the cell membrane but tend to occur upon receptor clustering in specific microdomains (6). DDR1 is not an exception and receptor aggregation has been suggested to participate in its activation (5). Our hypothesis is that in K-Ras-driven lung adenocarcinoma specific membrane microdomains may result in DDR1/Notch clustering facilitating their reciprocal activation. Furthermore, Ras signal output is qualitatively and quantitatively dictated by its own membrane sub-localization (7). Whether K-Ras^{G12V} might also be present in such microdomains remains to be determined, but in any case DDR1/Notch local enrichment may favour some post-translational modifications required for their full activity. As an illustrative example, Src is required both for full DDR1 activity as well as for the proteolytic activation of Notch in

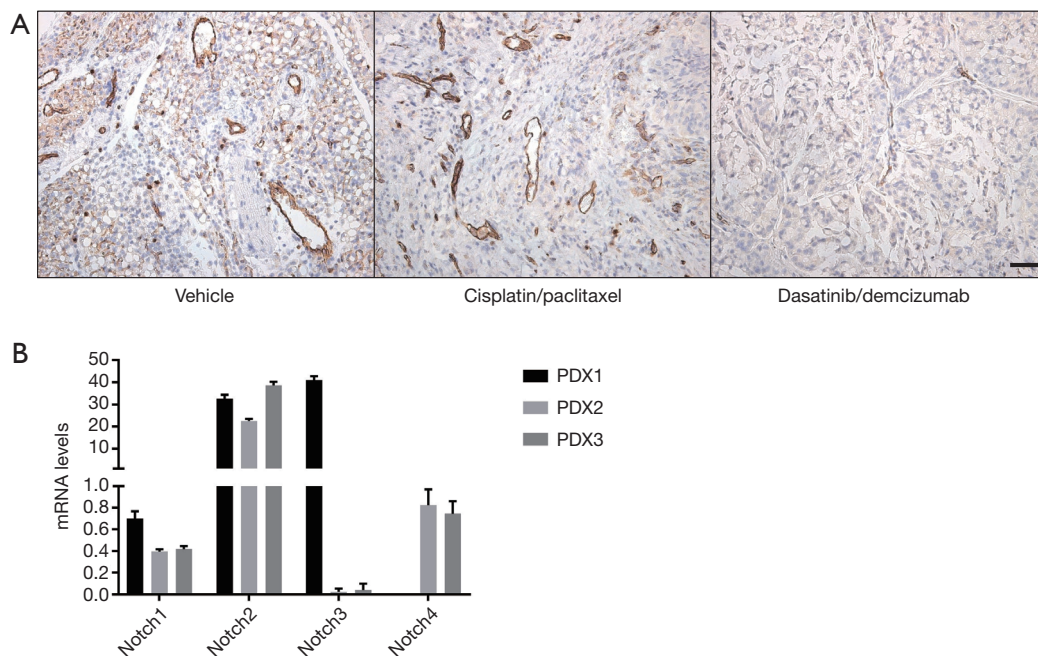


Figure 1 Effect of DDR1/Notch co-inhibition in the vasculature of K-RAS-driven lung adenocarcinoma & expression of Notch isoforms in this tumour type. (A) Representative immunostaining showing the CD31 endothelial marker in sections obtained from human lung adenocarcinoma (PDX). Tumours were orthotopically implanted in recipient mice, divided in three cohorts and treated in parallel with vehicle, cisplatin/paclitaxel chemotherapy or the combination of dasatinib/demcizumab. Samples were collected upon completion of the treatments. Scale bar: 100 μ m; (B) qRT-PCR analysis of Notch1–4 levels in a subset of three human lung adenocarcinoma PDX.

certain cellular contexts.

This putative DDR1/Notch co-regulation may be particularly relevant in specific tumour sub-populations. Notch and DDR1 have been reported to play a pivotal role to maintain cancer stem cell (CSC) traits in K-Ras driven lung adenocarcinoma and breast cancer respectively (8-10). In this context both DDR1 and Notch are subject to common regulatory mechanisms such as those involving PKC function (9,10) that, incidentally, also controls K-Ras membrane localization (7), again reinforcing the hypothesis that local clustering might facilitate co-regulation. Interestingly, the pharmacological co-inhibition of DDR1/Notch in lung adenocarcinoma PDX driven by K-Ras mutations eliminates the most aggressive tumour component and considerably delays disease relapse (1). This observation could be compatible with the combined DDR1/Notch inhibition being particularly effective on CSCs.

The therapeutic efficacy of DDR1/Notch co-inhibition may not only be a consequence of its effect in the tumour proper but in additional components. In agreement with an important Notch function in endothelial cells, co-inhibition of DDR1/Notch in lung adenocarcinoma resulted in

diminished tumour vasculature as measured by CD31 staining (*Figure 1A*). Whether this is exclusively reliant on Notch inhibition or whether DDR1 also plays an important role in tumour endothelial cells remains to be determined. In any case it is likely that the decreased vascularization contributes to the extensive tumour necrosis observed upon DDR1/Notch inhibition (1).

Finally, in his recent commentary Dr. Lendahl speculated on whether the Notch response is quantitatively or qualitatively blunted by DDR1 inhibition. It will be important to identify whether any of the four Notch receptors plays a prominent role in K-Ras-driven lung adenocarcinoma. In our study, Notch2 was over-represented in our limited subset of human PDX samples (*Figure 1B*). Accordingly, in the TCGA dataset Notch2 is the most frequently deregulated (amplified or up-regulated) isoform in human lung adenocarcinoma. In any case, and as discussed above, we have no evidence of a vertical relationship between the DDR1 and Notch pathways. Instead, our experimental evidences rather favour the existence of a bi-directional interaction. Whether this synergy is limited to their activation on the cell surface or,

in addition, their respective downstream signals converge to sustain pathways essential for tumour progression is currently unknown. In this regard, the activation of the MAPK pathway, known to be essential for the development of K-Ras^{G12V}-driven lung adenocarcinoma, is only efficiently suppressed when both DDR1 and Notch are simultaneously targeted (1). Whether this synergistic effect can be extended to other signalling and/or pro-survival pathways downstream of oncogenic K-Ras remains to be elucidated.

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Footnote

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