TARGETING THE NOTCH PATHWAY: A POTENTIAL THERAPEUTIC APPROACH FOR DESMOID TUMORS

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Abstract

Objective: To investigate the antitumor effect of the γ-secretase inhibitor PF-03084014 in desmoid tumor (DT) models.

Methods: The expression of Notch pathway components were examined at different levels in DTs. We showed that Hes1 (a helix-loop-helix transcriptional repressor) is overexpressed in DT tumors compared to dermal scar tissue, and that PF-03084014 caused significant decreases in NICD (notch intracellular domain that interacts with the DNA binding factor CSL) and Hes1 expression in DT cell strains. PF-03084014 decreased DT cell migration and invasion and also caused cell growth inhibition in DT cell strains, most likely through cell cycle arrest. Gene array analysis showed an up-regulation of WISP2 (a secreted protein member of the CCN family that is a downstream component of the WNT signaling pathway) after treatment with PF-03084014. Combined gene array data and IPA showed that WISP2 possibly regulates Notch and WNT pathways through integrin (transmembrane receptors that mediate cell-cell and cell-extracellular matrix interactions).

Conclusions: Our findings suggest that the Notch pathway is an important driver of DT tumorigenesis and that treatment with PF-03084014 has significant antitumor activity against DTs, and may comprise an alternative strategy for DT treatment.

Conclusions

- Notch pathway is highly activated in DT tissues and cell strains and an overexpression of Hes1 in DT as compared to scar was observed
- PF-03084014 inhibits the Notch pathway in DT, chiefly by inhibiting NICD and Hes1 expression
- Notch pathway blockade contributed to inhibition of our DT cell growth most likely through growth arrest rather than apoptotic cell death
- Treatment with PF-03084014 reduced DT migration and invasion, supporting PF-03084014 as a potential DT therapeutic strategy
- DT antitumor effects of PF-03084014 may also be related to an overexpression of WISP2 that can regulate integrin which in turn reportedly induces the Notch and WNT pathway

Results

- Figure 1. Immunohistochemical imaging demonstrating representative levels of Notch pathway components evaluated in DT. Images were captured using a 4x and 20x objectives. Nuclear Hes1 expression is higher in DT than in dermal scar tissue specimens
- Figure 2. Notch pathway is also activated in DT cell strains. A subset of DT primary culture exhibited NICD and nuclear expression via western blotting. NICD expression was only observed in the nucleus
- Figure 3. PF-03084014 treatment seems to slightly increase non–cleaved Notch (Notch1) protein expression in desmoid cell strains
- Figure 4. A subset of DT primary culture is sensitive to PF-03084014. A) A dose-dependent decrease in NICD and Hes1 was shown after treatment with PF-03084014 in all cell strains. B) Growth inhibitory effects (72 days) were determined via MTS assays. All desmoid cell strains were sensitive to the effects of PF-03084014. * denotes statistically significant effects (P < 0.05)
- Figure 5. PF-03084014 induces G1 cell cycle arrest contributing to desmoid growth inhibition. Treatment with PF-03084014 resulted in a statistically significant G1 cell-cycle arrest in desm39b cell strain (27 days after treatment; graphs represent at least 3 independent experiments). Decreased G2 fraction was also noted
- Figure 6. No apoptosis induction was observed in desmoid cell strains after PF-03084014 treatment
- Figure 7. PF-03084014 decreases desmoid cell migration and invasion. Decreased DT migration and invasion in response to PF-03084014 treatment was identified using modified Boyden chamber assays. Average migration and invasion per cells are depicted graphically
- Figure 8. WISP2 is overexpressed after treatment with PF-03084014. The treatment with PF-03084014 induced WISP2 mRNA expression
- Figure 9. Notch and WNT pathway cross-talk model. The green icons indicate downregulated genes and the red icons indicate upregulated genes, as result of PF-03084014 treatment

Table 1. Components of Notch Pathway Expression and Statistical Significance in Scar and Desmoid Tumors

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Nuclear component was analyzed. Nuclear component was analyzed

Acknowledgements

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*This work was conducted at MD Anderson Cancer Center. Danielle Braggio and Raphael Pollock are now at The Ohio State University.