

The Notch pathway : a new target in Gastrointestinal Stromal Tumors

Introduction

Gastrointestinal Stromal tumors (GIST) are the most common mesenchymal tumors in the gastrointestinal tract. A gain-of-function mutation of *KIT* plays a key role in the oncogenesis of these tumors. For patients with metastatic or unresectable GIST, the introduction of molecularly-targeted kinase inhibitors (imatinib, sunitinib) has significantly extended the lifespan of many patients with this disease. However the median time to progression on imatinib in GIST is 18-24 months. For those patients, there is an urgent need for new approaches to counteract resistance.

Notch signaling plays a critical role in the normal development of many tissues in maintaining the balance between cell proliferation, differentiation and apoptosis. Interestingly, the function of Notch signaling in tumorigenesis could be either oncogenic or antiproliferative, as a function of the context. The role of Notch signaling in GIST remains unknown but knowing that the cell of origin of GIST exhibits cellular features, suggesting that the tumor is of neuroectodermal origin, we hypothesized that Notch signaling could be an important pathway in GIST.

Methods

Cell culture

GIST-T1 (KIT exon11 del V560-Y579)

GIST882 (KIT exon13 mut K642E)

GIST48IM (KIT exon11 mut 560D and exon 17 mut D820A)

Lentiviral transduction

Flow cytometric analysis for GFP, Notch-PE, KIT-PE

RNA purification and cDNA synthesis

Real-time RT-PCR for *Notch1*, *Hes1*, *KIT*

Analysis of Cell Proliferation by:

MTS and automated Vi-Cell Analyzer

Patients and Tumor Specimens

GIST specimens obtained from patients IRB approved phase II study of neoadjuvant and adjuvant imatinib at MD Anderson

Statistical analysis

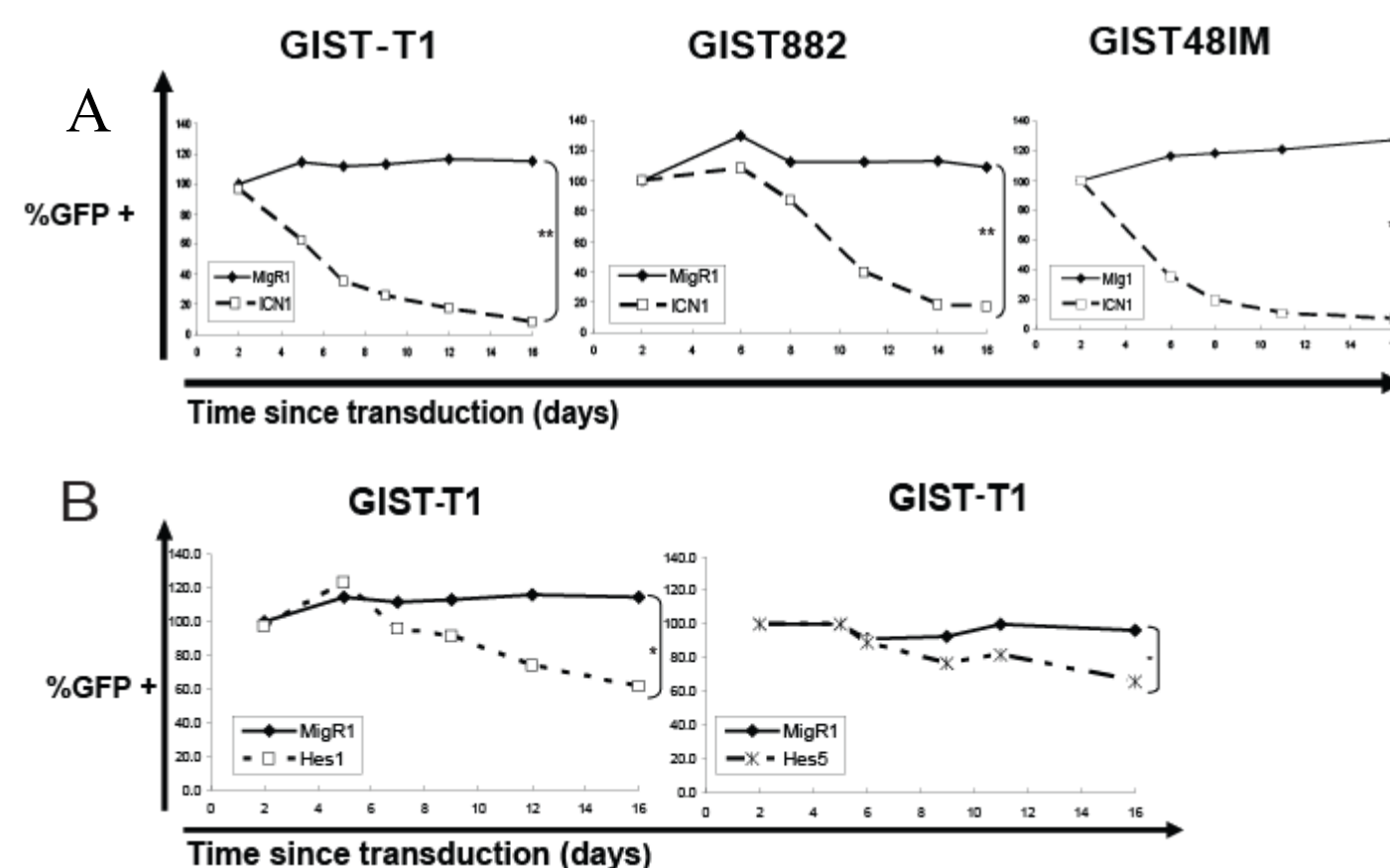
Student-t test, Kaplan-Meier analysis and log-rank tests

A value of $P < 0.05$ was considered significant.

Results

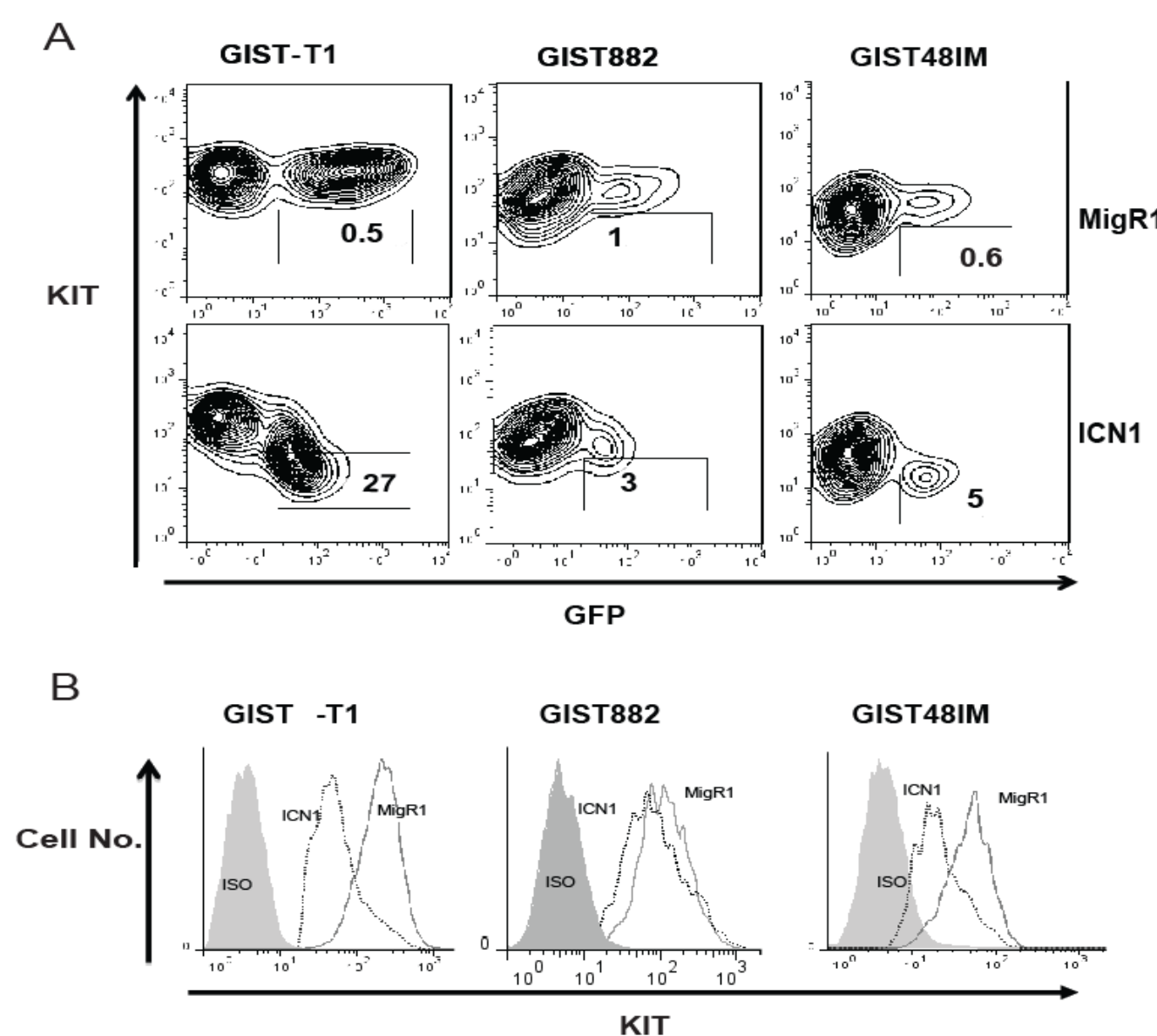
Intracellular *Notch1*, *Hes-1* and *Hes-5* transduction inhibit GIST cell growth

GIST cell lines were transduced with retroviral constructs expressing either a constitutively active truncated intracellular portion of Notch-1 receptor (ICN-1), Notch target genes *Hes-1* and *Hes-5*. The percentage of GFP+ cells at different times after transduction was an indirect measurement of the cell growth (figure 1).



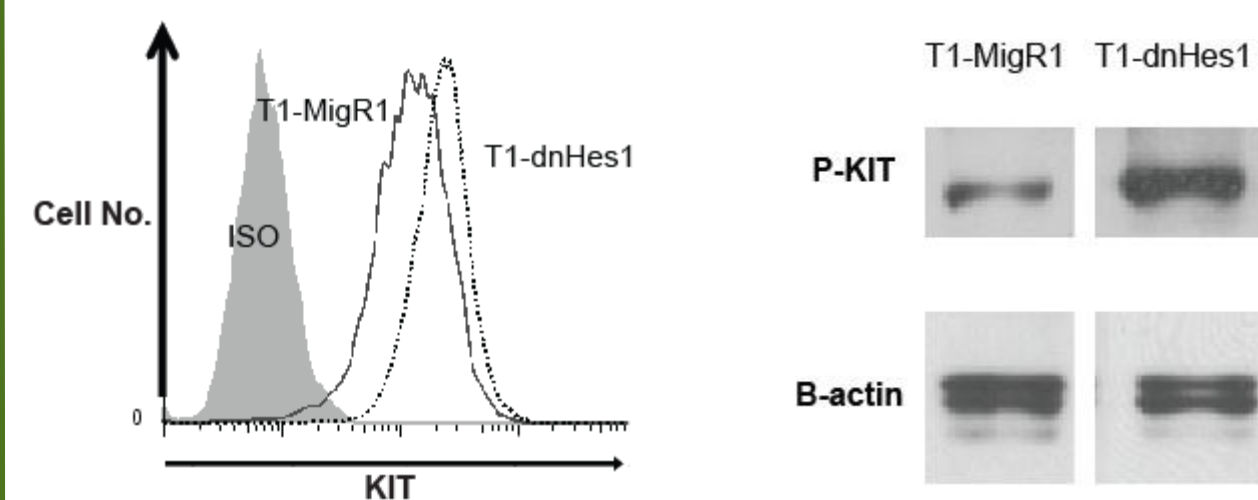
ICN1 transduction decreases KIT protein expression in GIST cells

Comparison of the KIT expression in the GFP+ cells transduced with vector (MigR1) alone or ICN1 (figure 2).



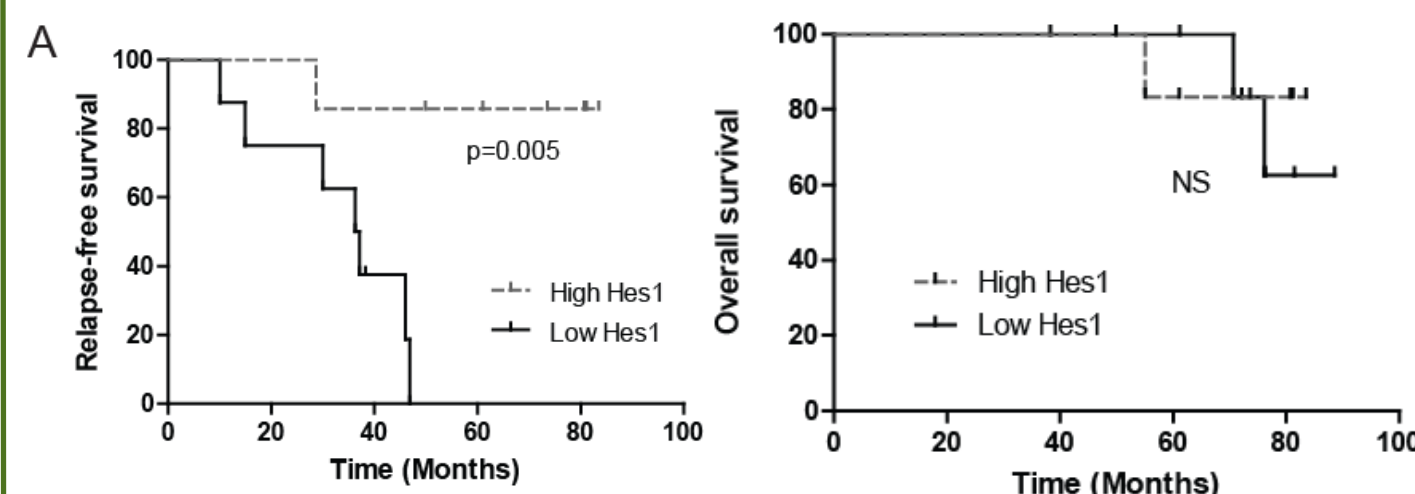
Transduction with dnHes1 increase KIT protein expression

The expression of KIT by flow cytometry and the phosphorylated form of KIT by western blotting was found to be higher after transduction of dominant negative Hes1 (figure 3).



GIST patients with high levels of mRNA *Hes1* expression have a longer RFS than patients with low levels of this expression

Kaplan-Meier analysis for relapse-free survival (RFS) and overall survival in GIST patients with high versus low *Hes1* mRNA expression (n = 15) (figure 4).



Conclusion

Despite the fact, that imatinib has dramatically changed the history of the disease; late resistances mainly caused by second mutations of *KIT* are important clinical challenges for GIST patients.

In the current study, we shown that activation of Notch-1 signaling leads to reduced cell viability and KIT expression in GIST cells. Additionally, mRNA *Hes1* expression was a prognostic factor for relapse in patients. All together, Notch pathway represent an appealing target in GIST.