Human Multiple Myeloma Cell Growth In Vitro and Bone Marrow Invasion In Vivo is Mediated by Notch System Modulation of the CXCR4/SDF1 Axis

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Multiple myeloma (MM) is caused by malignant plasma cells accumulating in the bone marrow (BM).

Interaction of MM cells with the BM stroma promotes tumor growth, metastasis, drug resistance, and is the main cause of morbidity and mortality.
BACKGROUND

• The chemokine receptor CXCR4 and its ligand stromal-derived-growth-factor-1 (SDF1) are critical regulators of the interaction between MM cells and BM stroma.

• MM cells frequently hyper-express CXCR4 and respond to SDF1, enhancing tumor infiltration[1], proliferation and osteolysis[2].
BACKGROUND

• Notch receptors similarly promote MM cell growth[3], drug resistance[4], and the associated osteolytic process[5].

• Notch pathway regulates CXCR4 expression and SDF-1α–driven chemotaxis in MM cells grown in vitro[6].
BACKGROUND
NOTCH SIGNALLING PATHWAY

• Notch gene mutation in Drosophila in 1913 by Dexter [7].
• Highly evolutionarily conserved cell signaling system important in [8,9]:
  – Cell–cell communication
  – Gene regulation of differentiation processes during embryonic and adult life
  – Maintenance of stem cell populations
  – Determination of cell fate decisions
  – Regulation of proliferation and apoptosis
  – Associated with angiogenesis
Hypothesis

• We hypothesized that the Notch pathway modulates the CXCR4/SDF1 axis in MM and regulates MM cell-bone marrow (BM) stromal interactions and BM invasion *in vitro* and *in vivo*.
OBJECTIVE

• Investigate how the cross-talk between the Notch system and CXCR4/SDF1 axis affects malignant plasma cell growth, survival, migration (in vitro) and bone invasion (in vivo).
METHODS

• We used gamma-secretase XII inhibitor (GSI-XII) to modulate Notch pathway in myeloma cells.

• Effects on Notch pathway on CXCR4 and SDF1 expression, cell cycle progression, apoptosis and migration were measured by qRT-PCR, ELISA and flow-cytometry.

• The CXCR4/SDF1 axis was alternatively stimulated by exogenous SDF1 or inhibited by anti-SDF1 blocking antibody.

• The effect of Notch inhibition on BM invasion was assayed in xenografted mice.
GSI-XII REDUCED THE EXPRESSION OF CXCR4 AND SDF-1

qRT-PCR assessment of GSI-XII effects on the expression of HES-1, CXCR4 and SDF-1 in MM cell lines. Bars indicate mean values calculated from 3 independent experiments (error bars represent SD).
GSI-XII REDUCED SDF-1 SECRETION BY MM CELLS

- E.L.I.S.A. assay for SDF-1 in the 48-h conditioned medium. Bars indicate mean values calculated from 3 independent experiments (error bars represent SD; *, unpaired t-test p<0.05; **, unpaired t-test p<0.01).
OPM-2 cells were cultured for 6 days with escalating doses of anti-SDF-1 blocking antibody or isotypic control. Viability was evaluated by MTT colorimetric assay (error bars: ±SD. t-test p: *, <0.05; **, <0.01).
MM cells were treated with 6 μM GSI-XII alone or combined with 0.5 μg/mL human recombinant SDF-1. A) cell growth. Error bars: ±SD. ANOVA and Tukey’s post-test p: *,<0.05; **,<0.01. B) cell cycle. C) Apoptosis. All flow-cytometry plots are representative of 3 experiments with similar results.
EVALUATION OF THE EFFECTS OF NOTCH SYSTEM INHIBITION IN AND IN VIVO MODEL OF MM
GSI-XII INHIBITS BONE MARROW INVASION BY MM CELLS

GSI-XII treatment affects MM cells spread and bone infiltration. (A) Tumor cells administered by i.v. xenograft in nude mice were identified as GFP+ cells. Among evaluated organs, the percentage of GFP+ events was significantly lower in GSI-XII compared with untreated mice only in the BM. This may indicate that Notch inhibition blocked CXCR4-driven localization in the bone marrow, where high amounts of SDF-1 are produced. (B) Analysis of cells staining positive for the active Notch-1 form and CXCR4 indicated that GSI-XII significantly reduced the frequency of MM cells in the bone marrow. Specificity of the assay was confirmed by the failure to detect positive events in tumor-free mice (data not shown).
Indicated MM cell lines were treated with DMSO or 6 mM GSI-XII for 48 h, then allowed to migrate in response to 100 ng/ml human recombinant SDF-1 in a standard transwell assay. Migration ability was measured by the migration index. Bars indicate mean migration index calculated from three independent experiments; statistical analysis was performed by analysis of variance and Tukey’s post-test (error bars, ±95% CI; *Po0.05; **Po0.01).
SUMMARY OF RESULTS

• Notch inhibition by GSI-XII reduced CXCR4 and SDF1 expression by MM cells.
• Notch inhibition by GSI-XII blocked MM cell chemotactic and proliferative response to SDF1.
• Interfering with Notch activity dramatically reduced the frequency of CXCR4-positive MM cells and hampered tumor ability to infiltrate the bone marrow.
DISCUSSION

1. Our results show a clear relationship between the Notch system and the SDF-1/CXCR4 pathway in MM.

2. Notch may play a key role in MM bone marrow invasion and progression by deregulating SDF-1/CXCR4 chemokine networks.

3. If our data are confirmed, the Notch/SDF-1/CXCR4 axis may emerge as both a prognostic indicator and a therapeutic target in MM.
CONCLUSION

• The Notch system regulates the CXCR4/SDF1 axis and bone marrow invasion in human MM.

• Notch-tailored therapies may effectively block CXCR4-mediated bone infiltration and associated lesions in MM patients, holding promise to improve patient outcome and survival.
REFERENCES


[6] Apicella et al., The Notch Meeting 2010


## ACKNOWLEDGEMENTS

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