Notch Signaling in Osteosarcoma

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Background

• Osteosarcoma (OS) is the most common primary bone cancer
• Treatments haven’t changed in the last 3 decades
• 5-year survival rate is 0-29% if metastases present
• Genetic syndromes have identified OS predisposition genes (TP53, RB1, WRN, RECQL4)
Physiological Notch signaling

Signal Sending Cell

Signal Receiving Cell

Ligand: JAG1, JAG2, DLL1, DLL3, DLL4, DLK1, DLK2

Cytoplasm

Nucleus

ADAM Protease (S2 site)

Gamma-Secretase (S3/4 site)

NECD

NICD

SEL10

CDK8

MAML

Fringe - Glycosylation

Furin - S1 Cleavage

OFUT1 - fucosylation

Canonical target genes (Hes1, MYC, DTX1, Hey1, Nrarp)

Cytoplasm

ER

Nuclear Notch signaling

Canonical target genes (Hes1, MYC, DTX1, Hey1, Nrarp)
Context-dependent Notch function during bone development

Mesenchymal Progenitors → Pre-Osteoblasts

Runx2

Notch → Osterix

Pre-osteoclast → OPG

Mature Osteoblasts → Osteocytes

LOW BONE MASS (Haju-Cheney)

HIGH BONE MASS

Alagille syndrome, SCD

Engin et al, Nature Medicine 2008; Tao et al. JBMR 2010
Increased Notch signaling in human OS & inhibition delays progression in mouse xenograft model

Engin et al., Lee Human Molecular Genetics 2009
Osteosarcoma formation and survival in NICD GOF mice

A
Rosa-NICD knock-in: 

\[ \text{Col1a1-2.3Cre} \quad \text{Col1a1-3.2CreER} \]

Jianning Tao et., B. Lee Cancer Cell 2014
Notch\textsuperscript{GOF} mouse OS models human OS

69% long bone in mouse OS

70% long bone in Human OS

Mouse OS
- Forelimb: 24%
- Tibia: 12%
- Femur: 33%
- Other: 31%
- Spine: 7%
- Hip: 10%
- Rib: 13%
- Skull: 2%

Summary Plot

538k x 61 bp

Chromosome
Notch activation together with p53 loss significantly accelerates tumor initiation and progression

Development of p53 OS is independent of Notch canonical pathway

Survival Rate vs. Time (days)

- p53cKO-RbpjcKO (n=32)
- p53cKO (n=35)

P=0.411
Cancer Cells in p53 OS models arise at a variety of stages during MSC differentiation.
Do human OS show evidence of Notch activation?

Microarray data set from 34 OS patient samples (Paoloni et al., 2009)
NOTCH1 mutations in human osteosarcoma (N=60)

NOTCH1

EGF LIN HD T RAM ANK TAD-PEST

NEC

2555 aa


I2550V 7648A>G Exon: 34 ID: #25

Jianning Tao
miR-34c regulates skeletal homeostasis

BMP-2

miR-34c
Osteoblasts

Notch signaling
Notch1
Notch2
Jag1
Hey1

Satb2
Runx2
Osx
Col1a1
Ocn

Osteoclastogenesis
Osteoblastogenesis

Bone homeostasis

Yangjin Bae et al. Human Molecular Genetics 2012
Correlation of Notch1/2 and Jag1 with miR-34c expression and metastatic potential in OS cell lines.
Inhibition of hOS progression by miR-34c in a xenograft model

143b (p53-/-):hOS

Tumor size

Relative expression of miR-34c (log(RQ))

Control  TRC  miR34C

0  1  2  3

Tumor vol. (mm³)

0.0  0.5  1.0  1.5  2.0  2.5

3wks  4wks

Yangjin Bae
Expression of miR34c improves survival of Col1a1Cre;p53^{f/f} osteosarcoma mice

Expression of miR34c improves survival of Col1a1Cre;p53^{f/f} osteosarcoma mice.

P = 0.03

OB^{P53/- ; miR-34c (n=37)}

OB^{P53/- (n=36)}
Lung metastasis in p53-/- OS is decreased by miR34c GOF

- OB $^{p53-/-}$: 38.5%
- OB $^{p53-/-}$; miR-34c: 8.3%

With Dr. Frank Gannon
Putative miR-34c targets associated with lung metastasis

Genes up-regulated in lung metastases and primary OS in sleeping beauty forward genetic study

Putative miR-34c targets:
TargetScan, miRWalk, miRanda, RNA22

Downregulated in miR-34c highly expressed cells

Moriarty et al., *Nature Genetics* 2015
Putative targets-CD2AP and TLK1 are higher in the metastatic hOS cell line.

![Graph showing fold change of CD2AP and TLK1 in different cell lines with miR-34c levels](image-url)
miR-34
(Upstream)

Notch

(Downstream Targets)

Genome instability

OS

Metastasis

OS (Genome instability) - Metastasis - Notch - miR-34 (Upstream)

(Downstream Targets)
Summary

- Notch1 gain of function in committed osteoblastic lineage cells is sufficient to cause murine osteosarcoma

- Notch activation combined with p53 loss synergistically accelerates osteosarcoma development in mice, though p53 driven osteosarcoma is not dependent on canonical Notch signaling

- Missense mutations of NOTCH1 in human primary osteosarcoma specimens

- miR34c regulates osteoblast and osteoclast differentiation and Notch signaling in osteosarcoma

- Gain of miR34c leads to increased survival and decreased lung metastasis in the p53-/- model of OS

- Forward genetic screens in mouse p53-/- OS in combination with human WES and WGS identifies potential novel miR34c targets as well as candidate genetic drivers
Breast cancer bone metastasis & Notch signaling

Col1a1-Jag1 mice show elevated osteoclastogenesis and cortical remodeling

Increased osteoclasts

TRAP staining
Yangjin Bae
Increased breast cancer cell engraftment in Col1a1-Jagged1 transgenic mice
Acknowledgements

Lee Laboratory
Feyza Engin*
Jianning Tao*
Yangjin Bae
Shan Chen
Huan Chang Zeng

BCM
Hui Zheng
Lisa Wang
Frank Gannon
Larry Donehower
Jason Yustein
Rui Chen

Princeton
Yibing Kang
Hanqui Zheng