Therapy in osteosarcoma: Biological Rationale for Future Studies

Results from the EuroBoNet consortium

Edited version
Annual EuroBoNeT meeting in Münster – Jan. 2010
• Developmental pathway components: **Wnt/\(\beta\)-catenin** signaling

• Tyrosine kinases: **Ezrin**

• Immune system: **Macrophages**
Wnt signaling in normal osteoblast development

- MSC → Skeletal MSC → RUNX2 → osteochondro progenitor → β-catenin
- IHH SOX9 → preosteoblast → DKK2 RUNX2 → osteoblast → DKK1 FOSL1 → osteocyte
- Genetic instability: Rb off, p53 off
- WNT on/off, IHH on/off, BMP on/off

chondroblast → GG PTHRP → chondrocyte
• Colorectal e.o. tumors: aberrant upregulation
WNT signaling is inactive in osteosarcoma

Cai et al., J Pathol 2010; 220: 24–33
Restoration of Wnt signaling by GSK3β inhibitor (GIN)

Luciferase activity

Axin2 mRNA expression
WNT signaling inhibits proliferation in OS
WNT signaling stimulates osteogenic differentiation

\[ \beta\text{-glycerophosphate}, \text{dexamethasone, ascorbic acid} \]
Conclusions on Wnt in osteosarcoma

- Canonical WNT is not constitutively active in osteosarcoma
- Activation of WNT can cause
  - Inhibition of proliferation
  - Stimulation of differentiation
- Stimulation of WNT may suppress osteosarcoma
Poor prognosis of metastasised OS
Ezrin

- Ezrin is a protein-tyrosine kinase substrate involved in adhesion, migration and cytoskeleton organisation.
- Mouse model microarray study identified ezrin as mediator for metastasis in osteosarcoma (Khanna, 2001).
- Ezrin expression associated with poor survival in osteosarcoma clinical samples (n = 19) (Khanna, 2004).
- Small molecule inhibitors for ezrin (AACR 2009).
Ezrin protein expression in a larger clinical cohort

N=144

Alex Mohseny, in preparation
Ezrin protein is not associated with survival

P = 0.7
Detection of differential expression (DE)

- Collection of osteosarcoma pré-CT biopsies from the EuroBoNet network of excellence
  - www.EuroBoNeT.eu

- Selection of groups
  - Patients developing metastases within 5yrs (n=34)
  - Patients without metastases within 5yrs (n=19)

- Genome wide expression profiling with Illumina Human-6 v2 BeadChips with 48,701 different probes

- Detection of differentially expressed genes (DE)
  - BH FDR-adjusted p-values <0.05
• 139 probes significantly DE

• High expression of macrophage-associated probes in patients who did not develop metastases within 5yrs
  • ~50% of all significantly DE probes (possible) macrophage-association
  • E.g.:
    • CD14 LPS receptor
    • CSF2RA colony stimulating factor 2 receptor

Marieke Kuijjer and Emmeline Buddingh*, in preparation
Tissue arrays with 144 tissues from 88 patients

B (n=73)

R (n=45)

39

21

11

10

2

3

10

M (n=26)

CD14
IHC staining for macrophages and vascular density

OS TMA with 145 samples from 88 patients

MoAbs Neomarkers

CD14

CD31

100 µm

nr of CD31+ vessels/core

nr of CD4+ cells/core

Q1 Q2 Q3 Q4

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Phenotype of macrophages in osteosarcoma

‘anti-tumor’

“Classical activation”

Higher expression of MHC molecules (incl HLA II)
Pro-inflammatory cytokines: IL-10^{low}/IL-12^{high}, TNF-α, IL-6
Reactive oxygen species
Activation of adaptive immune system
Expression of CCR7

‘pro-tumor’

“Alternative activation”

Anti-inflammatory cytokines: IL-10^{high}/IL-12^{low}
Pro-angiogenic VEGF, CXCL8, PDGF
Expression of matrix metallo-proteinases
Suppression of adaptive immune system
Expression of scavenger receptors eg CD163, MSR1
Double staining for macrophage subtype

Co-localisation tool in Nuance: % positive pixels per area region of interest

CD14 CD163 double positive

CD163 single positive

CD14 single positive

M1, antitumor, HLAII – M2, protumor, CD163
• Liposomal Muramyl Tripeptide, L-MTP-PE, Mepact, Mifurmatide
• Activation of monocytes and macrophages
• Successful trial in xenografts and dogs
• Phase III trial improved survival from 70 to 78% in primary localized disease
Our findings that macrophages are associated with less metastases and better survival provide a rationale for applying MTP-PE as adjuvant treatment for osteosarcoma.

Monitoring efficacy of treatment:

- Pre-treatment biopsies
- Post-treatment biopsies
- Peripheral blood

- CD14
- IL10 (M2)
- IL12 (M1)
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Emmeline Buddingh’
Jakob Anninga
"We came up with a pill that cures everything...but I'm warning you, it's gonna be expensive."
Tumor associated macrophages

Genome-wide expression profiling

Illumina Human-6 v2 BeadChips

Variance stabilizing transformation and robust spline normalisation

Removal of outlier arrays: arrayQualityMetrics
No expression of macrophage-associated genes *HLA-DRA* and *CD14* by 19 osteosarcoma cell lines