New chondrosarcoma cell lines and mouse models to study the link between chondrogenesis and chemoresistance

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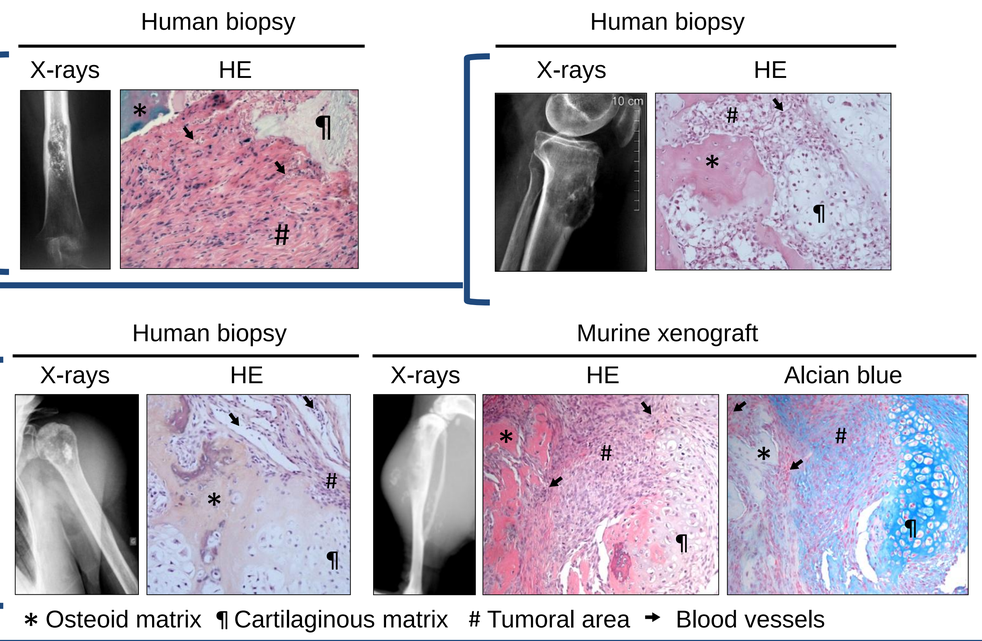
INTRODUCTION

Chondrosarcomas are cartilage-forming, poorly vascularized tumors. They represent the second malignant primary bone tumor of adults after osteosarcoma but in contrast they are resistant to chemotherapy and radiotherapy, surgical excision remaining the only therapeutic option. Few cell lines and animal models are available, and the mechanisms behind their chemoresistance remain largely unknown. Our goal was to establish new cell lines and animal cancer models from human chondrosarcoma biopsies to study their chemoresistance.

RESULTS

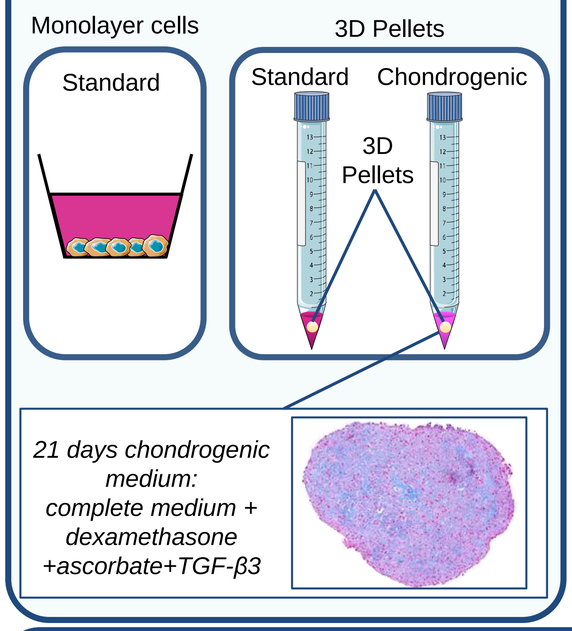
1. From 10 chondrosarcoma biopsies, three tumoral cell lines and two animal models in nude mouse were established.

Patient	Gender	Age	Sub-type	Cell line	TP53	IDH1	IDH2	p16^{INK4A}	Tumorigenicity
BCSCH01	M	83	Conventional central	No	-	-	-	-	Yes
BCSCH03	F	74	Dedifferentiated	Yes	c.318delC	WT	WT	Deletion	No
BCSCH06	M	57	Conventional central	No	-	-	-	-	-
BCSCH30	M	46	Secondary	No	-	-	-	-	-
BCSCH34	M	73	Conventional central	Yes	WT	p.R132C	WT	Deletion	No
BCSCH36	F	28	Secondary	No	-	-	-	-	-
BCSCH37	F	41	Secondary	No	-	-	-	-	-
BCSCH45	F	64	Conventional central	No	-	-	-	-	-
BCSCH56	F	64	Conventional central	Yes	WT	WT	p.R172S	Deletion	Yes
BCSCH59	F	67	Conventional central	No	-	-	-	-	-

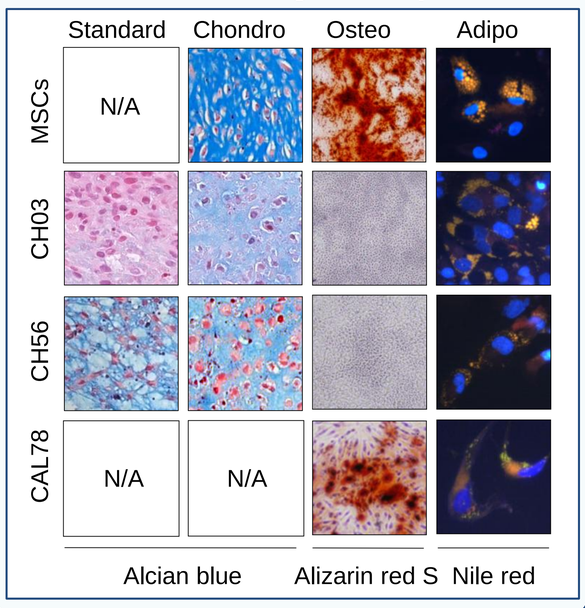


METHODS

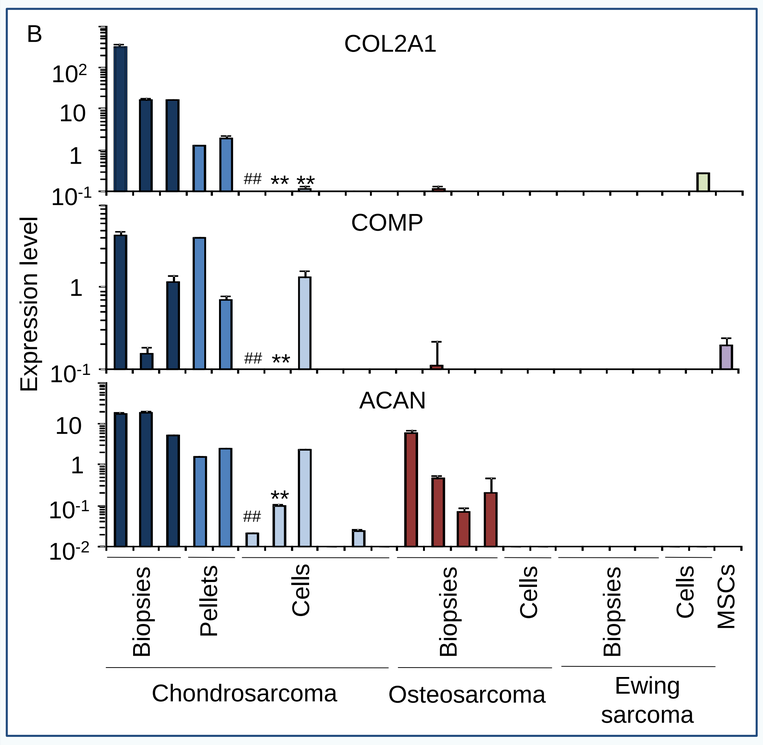
Three culture conditions were used:

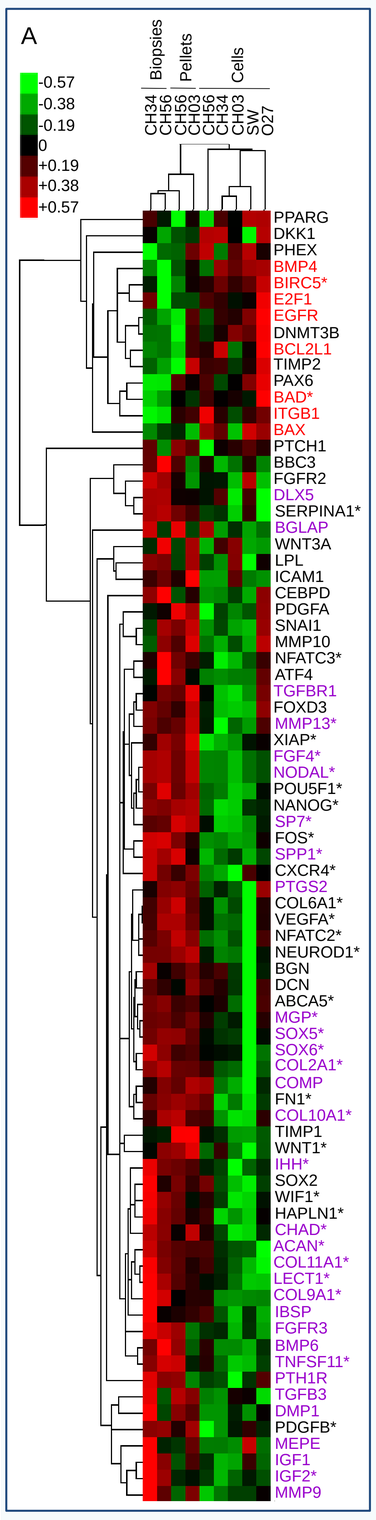


2. The new chondrosarcoma cell lines are able to synthesize cartilaginous matrix in chondrogenic 3D pellets.



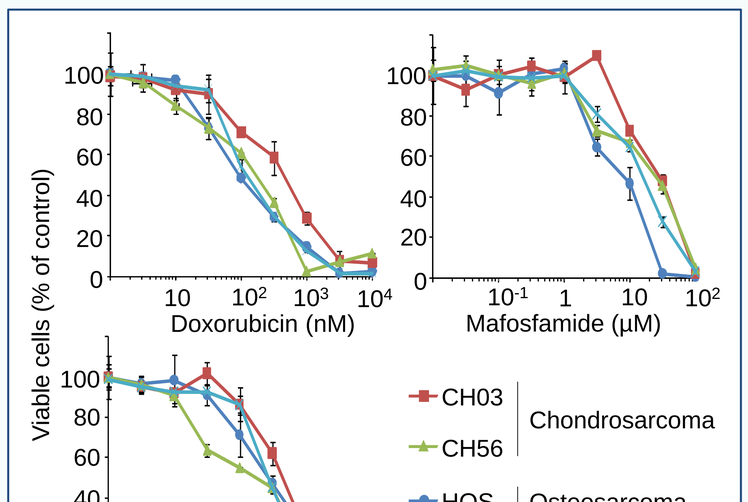
3. Chondrosarcoma cells express high levels of cartilage markers in chondrogenic 3D pellets compared to standard monolayer culture.





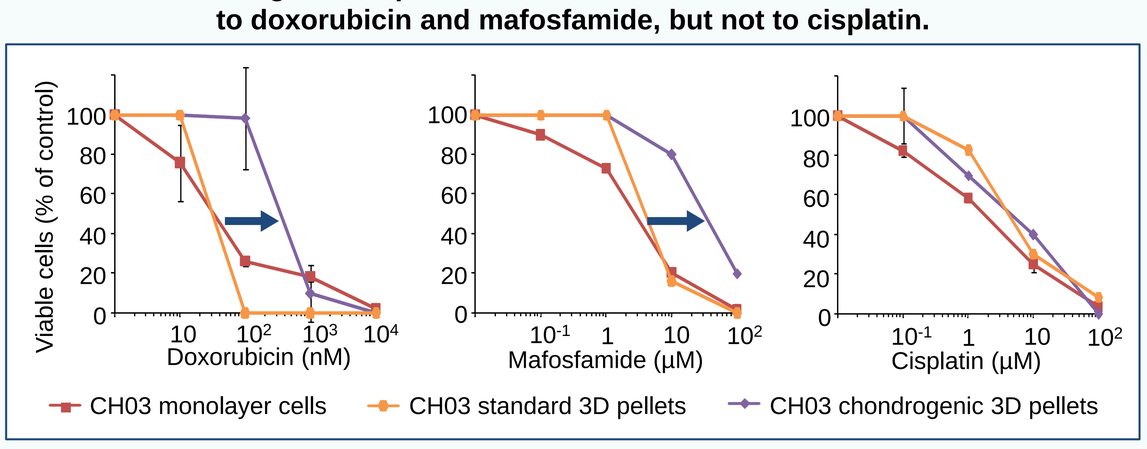
*A: TaqMan® Low Density Array: screening of upregulated genes in chondrosarcoma biopsies and chondrogenic 3D pellets (red, apoptosis/cell cycle; purple, cartilage; *p<0.05, monolayer VS biopsies and pellets*
*B: Quantitative RT-PCR: expression of three cartilage markers in chondrogenic 3D pellets (**p<0.01, compared with the original biopsy, ;## p<0.01 compared with chondrogenic 3D pellets)*

4.Chondrosarcoma cells cultured as monolayer are sensitive to doxorubicin, mafosfamide and cisplatin.



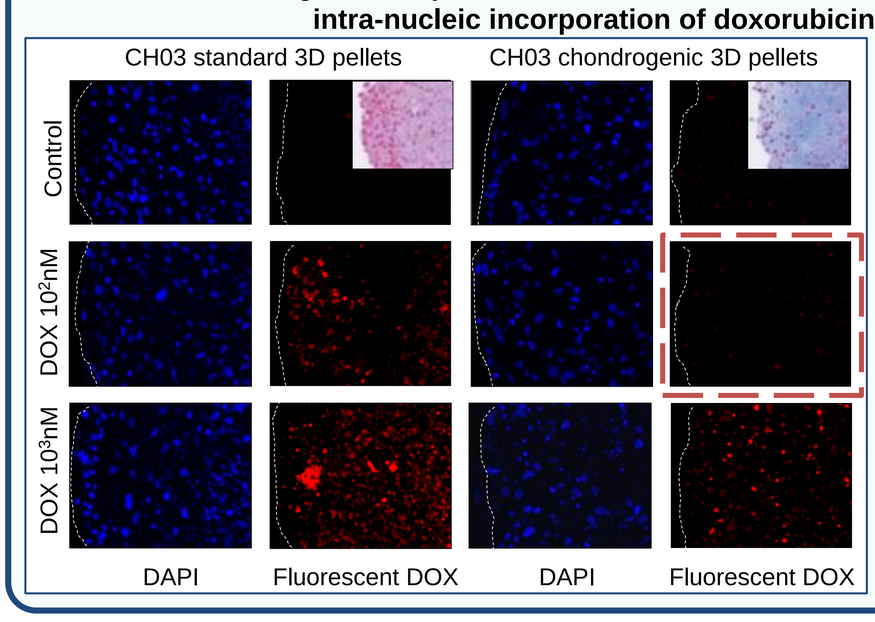
Viability XTT assays: sensitivity of the two new chondrosarcoma cell lines cultured as monolayer to three chemotherapeutic agents compared to osteosarcoma and Ewing sarcoma cell lines (3 day treatment).

5. In chondrogenic 3D pellets, chondrosarcoma cells are more resistant to doxorubicin and mafosfamide, but not to cisplatin.



Viability trypan blue assays: sensitivity of CH03 chondrogenic 3D pellets to three chemotherapeutic agents compared to standard 3D pellets and monolayer cells (3 day treatment).

6. In chondrogenic 3D pellets, chondrosarcoma cells have an impaired intra-nucleic incorporation of doxorubicin.



Accumulation of doxorubicin (DOX): fluorescence microscopy on 3μm sections of standard and chondrogenic 3D CH03 pellets treated with DOX for 3 days. Insets:alcian blue staining of untreated 3D pellets.

CONCLUSION

These results indicate that the cartilaginous matrix produced by chondrosarcoma cells may impair diffusion of chemotherapeutic drugs based on their hydrophobicity/water solubility and thus contribute to chemoresistance. Although they lack angiogenesis and metastasis development, these chondrogenic 3D pellets represent valuable models for this complex pathology, especially to study the link between chondrogenesis and chemoresistance.