ANALYSIS OF THE MOLECULAR MECHANISM IN METASTASIS OSTEOSARCOMA USING GENE EXPRESSION RE-ANALYSIS

Rawikant Kamolphiwong[1], Kanyanatt Kanokwiroon[1*], Weerinrada Wongrin[2], Parunya Chaiyawat[3,4], Jeerawan Klangjorhor[3,4], Dumnoensun Pruksakorn[3,4*]

[1] Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand
[2] Department of Statistics, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
[3] Musculoskeletal Science and Translational Research Center, Department of Orthopaedics, Chiang Mai University, Chiang Mai, Thailand
[4] Center of Multidisciplinary Technology for Advanced Medicine (CMUTEAM), Faculty of Medicine, Chiang Mai University, Thailand

Center of Multidisciplinary Technology for Advanced Medicine
CMUTEAM

ABSTRACT

Background: Survival rate of osteosarcoma has remained plateaued for the past three decades and mechanism of progression remain unclear. Therefore, our aim is to explore the mechanism and identify new therapeutic targets for metastasis osteosarcoma by using gene expression profile.

Materials and Methods: Metastasis osteosarcoma gene expression microarray data were retrieved from available database. Differential gene expression with logFC ≥ 4 (log fold change) and adjusted p-value < 0.05 were identified as primary candidate genes (PCG). PCGs were further used to find secondary candidate genes (SCG) which involved in pathways. PCGs and SCGs were matched with genes target from the Drug repurposing hub. Finally, expression of potential genes and pathway were explored by western blotting.

Results: Eighty-one genes were identified as PCG which 4 genes were able to match with 6 drugs. Sixty genes corresponding to top ten pathways were identified as SCG. However, only 3 pathways (negative regulation of anoikis, regulation of anoikis, surfactant metabolism) with 8 genes were matched with 77 Drugs. CAV1 and CAV2 were identified as PCG and found in anoikis resistance pathway which may play an important role in metastasis disease including osteosarcoma. We found that the CAV1 and CAV2 proteins were down regulated in 143B osteosarcoma cell seeded on poly-Hema treated wells (as anoikis resistance condition) and reversed back to the normal levels when the anoikis resistance cells were re-attached.

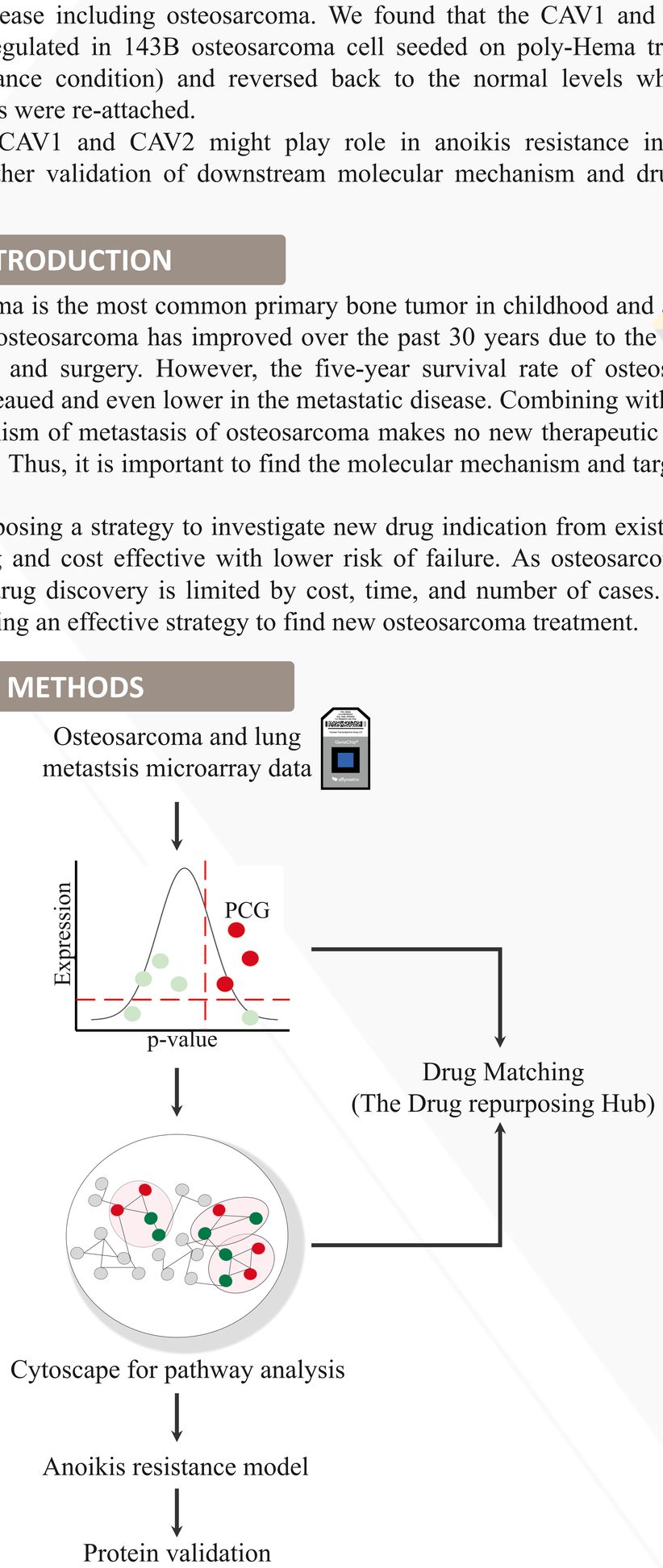
Conclusion: CAV1 and CAV2 might play role in anoikis resistance in osteosarcoma. However, further validation of downstream molecular mechanism and drug inhibition is needed.

INTRODUCTION

Osteosarcoma is the most common primary bone tumor in childhood and adolescence. Treatment of osteosarcoma has improved over the past 30 years due to the treatment of chemotherapy and surgery. However, the five-year survival rate of osteosarcoma has remained plateaued and even lower in the metastatic disease. Combining with the unclear of the mechanism of metastasis of osteosarcoma makes no new therapeutic treatment to be discovered. Thus, it is important to find the molecular mechanism and targets for drug discovery.

Drug repurposing a strategy to investigate new drug indication from existing drugs. It is time saving and cost effective with lower risk of failure. As osteosarcoma is a rare disease new drug discovery is limited by cost, time, and number of cases. That makes drug repurposing an effective strategy to find new osteosarcoma treatment.

METHODS



RESULT

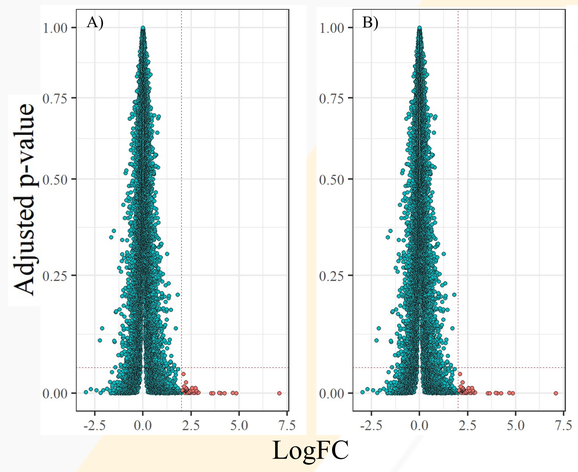


Figure 1 Differential gene expression

Affymetrix microarray HG-U133A and Hugene-1.0 platform with the samples of osteosarcoma and lung metastasis osteosarcoma were chosen for re-analysis. Forty-two and 39 from HG-U133A and Hugene-1.0 were identified as primary candidate genes (PCG) (Figure 1). Then PCGs were further enriched to find pathways related to osteosarcoma metastasis. Genes involved in the pathways were identified as secondary candidate genes (SCG). The top 10 pathways, PCGs and SCGs are shown in Table 1.

PCGs and SCGs were then further matched with drugs target downloaded from The Drug Repurposing Hub. Seventy-seven drugs were able to target genes from negative regulation of anoikis, regulation of anoikis, surfactant metabolism as listed in Table 1.

Table 1 The top ten pathways related to osteosarcoma metastsis and drugs target

Pathways	SCG involved in pathways (PCG)	Druggable Pathway (Number of Drugs and examples)
Defective CSF2RA causes pulmonary surfactant metabolism dysfunction 4 (SMDP4)	CSF2RA, CSF2RB, SFTA3, SFTPA1, SFTPA2 (SFTPB, SFTPC, SFTPD)	No
Defective CSF2RB causes pulmonary surfactant metabolism dysfunction 5 (SMDP5)	CSF2RA, CSF2RB, SFTA3, SFTPA1, SFTPA2 (SFTPB, SFTPC, SFTPD)	No
Diseases associated with surfactant metabolism	CSF2RA, CSF2RB, SFTA3, CCDC59, SFTPA1, TTF1, SFTPA2 (SFTPB, SFTPC, SFTPD, SLC34A2)	No
Membrane raft assembly	FLOT, FLOT2, CAV3, ANXA2, S100A10 (CAV1, CAV2, EMP2)	No
Surfactant metabolism	CKAP4, ADORA2A, CSF2RA, CSF2RB, ADRA2A, DMBT1, ADGRF5, SFTA3, GATA6, CCDC59, LMCD1, ZDHHC2, SFTPA1, TTF1, SFTPA2, ADRA2C, ADORA2B, P2RY2 (CTSH, SFTPB, SFTPC, SFTPD, SLC34A2)	Yes (64 drugs; risperidone, carvedilol, desipramine, epinephrine, nortriptyline Adenosine, aminophylline, caffeine, etc.)
Negative regulation of anoikis	NOTCH1, PTRH2, PTK2, SRC, ITGA5, ITGB1, PIK3CA, NTRK2, MCL1, BCL2 (CAV1, CAV2, CEACAM6, SNAI2)	Yes (14 drugs; docetaxel, paclitaxel, rasagiline, venetoclax, copanlisib, idelalisib, bosutinib, dasatinib, ponatinib, vandetanib, etc.)
Negative regulation of protein autophosphorylation	ZBTB7C, ENG, LRP1 (CAV1, CAV2)	No
Regulation of anoikis	PTENP1, CHEK2, PELP1, NOTCH1, PTRH2, PTK2, NTRK2, ITGA5, ITGB1, MCL1, BCL2, PIK3CA, SRC (CAV1, CAV2, CEACAM6, SNAI2)	Yes (14 drugs; docetaxel, paclitaxel, rasagiline, venetoclax, copanlisib, idelalisib, bosutinib, dasatinib, ponatinib, vandetanib, etc.)
Membrane raft organization	FLOT1, CAVIN3, FLOT2, CAV3, S100A10, ANXA2 (CAV1, CAV2, EMP2)	No
Extracellular matrix assembly	IHH, COL1A2, SOX9, NOTCH1, LOX, EFEMP2, FBLN5, TGFB1, SMAD3 (HAS2, MFAP4, RGCC)	No

We chosen negative regulation of anoikis or anoikis resistance pathway as it is an important pathway that play roles in metastasis with drug targets. Osteosarcoma cell line 143B were plated in poly-HEMA to develop the anoikis resistance cell. We found that CAV1 and CAV2 were downregulated during anoikis resistance (Figure 2A). Moreover, we found that the expression were increased when cells were re-attached (Figure 2B).

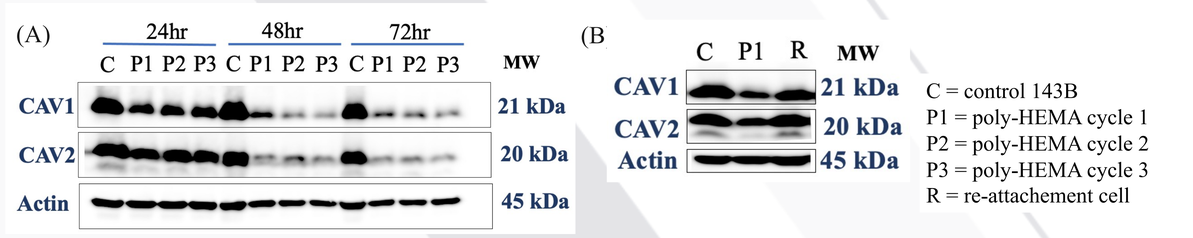


Figure 2 Protein expression level of CAV1 and CAV2 when plated in poly-HEMA at 24,48 and 72 hr. Actin was used as loading control.

CONCLUSION

This study we identified PCG and SCG and pathway related to osteosarcoma metastasis (Table 1). We also reported the potential therapeutic targeted to PCGs and SCGs, but further studies are required. Finally, we found that CAV1 and CAV2 may play an important role during the process of detachment or re-attachment. The role of CAV1 and CAV2 need to be explored.

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