

88 : The potential of Pim kinase as a candidate of target protein for new parasitocidal drug against *Echinococcus multilocularis*

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1. Life Cycle of *Echinococcus multilocularis*



Germinative stem cells in metacystode vesicles, which play key roles of their regeneration and longevity, are not very susceptible to Albendazole.

2. Human Pim1 and Echinococcal Pim kinase

Alignment of the kinase domain

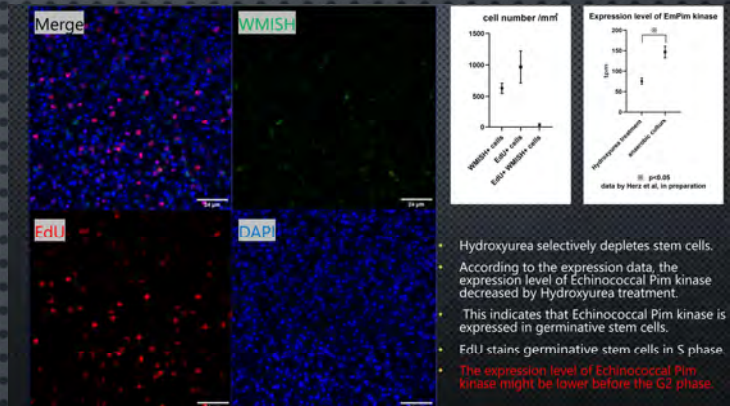
| | |
|-----------|--|
| Consensus | QDLFD I T E R G L E E R F F Q V A H C H G V H R I K D N L I D L G E K L I D F G S G A L L D Y T D F G T R |
| HsPim1 | V D L F D F T E R G A L Q E L A R S F F W V L E A V R H C H C E V L H R I K D N L I D L N R G E L K L T D F G S G A L L K T V Y T D F G T R 80 |
| HsPim3 | A D L L D F T E R G A L D E P L A R N R F A W L A V R H C H C E V H R I T K D N L I V L S E E L K L T D F G S G A L L K G T V Y T D F G T R 80 |
| HsPim2 | A D L L D Y I T E R G P L E G P S R C F F G V V A I Q H C S R G V V H R I K D N L I D L R R G C A K L I D F G S G A L L H D E P Y T D F G T R 80 |
| EmPim | V D L F D Y T C R G V L S E C E S A F I M Y G L I G I L L K H E A V L H R D L K D N L I T S D N H T Q L I D F G S G A T L H D I G Y N D F G G T R 80 |

Consensus: V Y S P P E W I T H Y H G S A V M S L G I L L Y D M V C G D I P F E D E I L G F R V S

| | |
|--------|---|
| HsPim1 | V Y S P P E W I R Y R Y H R S A A V V S L G I L L Y D M V C G D I P F E D E I L R G V F F R O R V S S 136 |
| HsPim2 | V Y S P P E W R Y H Y H R S A T V S L G I L L Y D M V C G D I P F E D E I L R G E L L R R K V S P 136 |
| HsPim3 | V Y S P P E W S R D Y H A T V S L G I L L Y D M V C G D I P F E D E I L E A L I F P A R V S P 136 |
| EmPim | V Y S P P E W K I N G Y R K S A L W I V G I L L D M I N D I P F S D H E L L S G A V F R T L V S 136 |

- Pim: Proto-oncogene serine/threonine protein kinase
- Positive regulator of G2/M transition
- HsPim1 activates M phase inducer Cell division cycle phosphatase 25C(Cdc25C)
- HsPim1 inactivates Cdc twenty-five C associated kinase(C-TAK1)
- In Echinococcal genome, there are Pim-like gene, Cdc25-like gene, and C-TAK1 like genes

3. In situ hybridization of Echinococcal Pim kinase



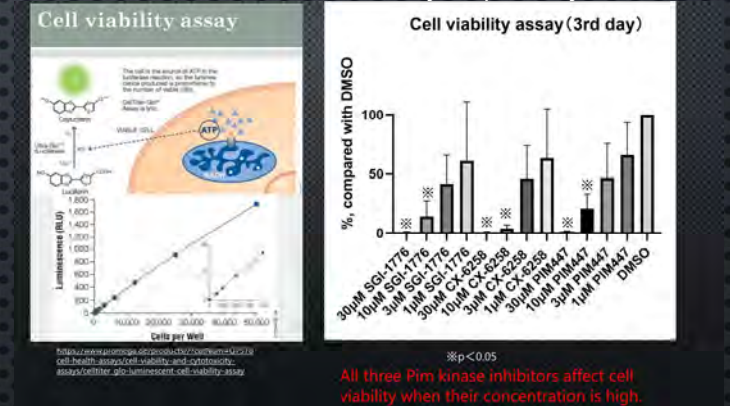
4. Interaction with Echinococcal Pim kinase, C-TAK1 and Cdc25

| pGBKT7 | Pim | Pim | p53 |
|-----------------|-------|-------|-----------|
| pGADT7 | Cdc25 | Empty | T Antigen |
| Optical density | 1.0 | 0.10 | 0.01 |

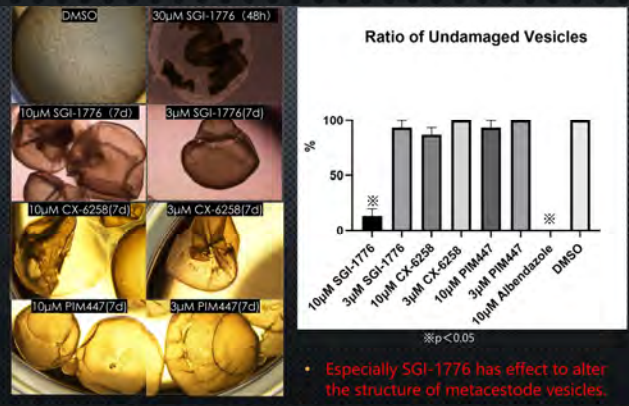
Y187 transformants of pGBKT7-C-TAK1 did not grow enough for the quantitative assay

Echinococcal Pim kinase seems to have interactions with Echinococcal Cdc25 and C-TAK1

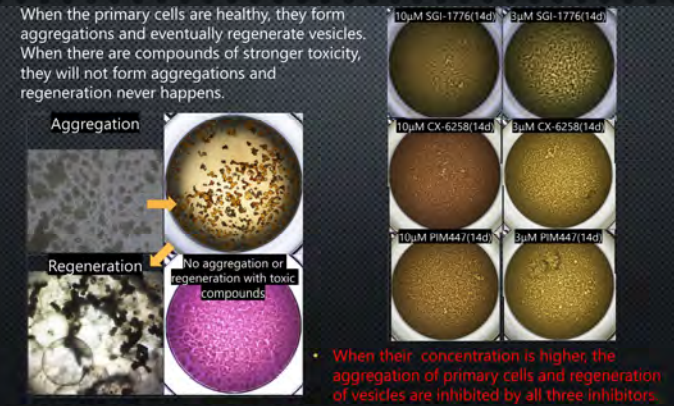
5. Effect on the cell viability of primary cells



6. Effect on Matured Vesicles



7. Effect on the vesicle regeneration



8. Discussion/Conclusion

- From the result of *in situ* hybridization(slide3), the expression level of Echinococcal Pim is not likely to be high in germinative stem cells of S phase. Its expression might be higher in G2 phase.
- The result of yeast-two hybrid(slide4) indicated that Echinococcal Pim kinase has interaction with Echinococcal Cdc25 and C-TAK1. The same as human Pim1, Echinococcal Pim kinase is likely to regulate cell cycle progression through the activation of Echinococcal Cdc25 and inactivation of Echinococcal C-TAK1.
- From the result of *in vitro* screening(slide 5-7), we can expect that the inhibition of Echinococcal Pim kinase has some effect on the proliferation of germinative stem cells, and eventually has effect on the survival or regeneration of larval vesicles.
- One Pim kinase inhibitor, SGI-1776 showed stronger detrimental effect on both larval vesicles and primary cells.
- Echinococcal Pim kinase can be a good target of parasiticides and Pim kinase inhibitors can be promising lead compounds.