Pim kinase inhibitors possess anticancer properties through downregulation of EGFR family in HER2-positive cancer cells

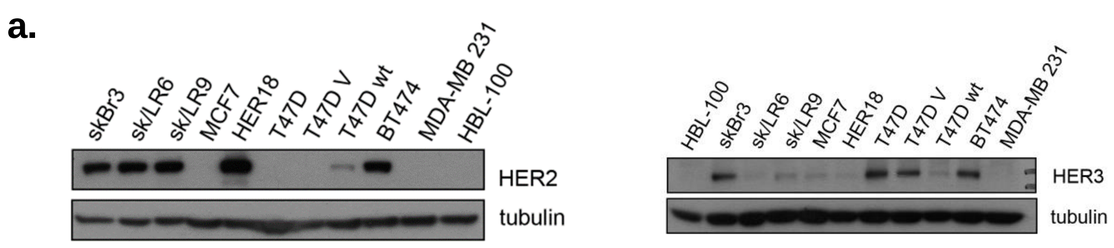
王于菲[1] ,王文玲[1], 黃偉謙[1,2,3,4]
Yu-Fei Wang[1], Wen-Ling Wang[1], Wei-Chien Huang[1,2,3,4]

[1] Graduate Institute of Cancer Biology, China Medical University, Taichung 404, Taiwan
[2] Center for Molecular Medicine, China Medical University and Hospital, Taichung 404, Taiwan
[3] The Ph.D. Program for Cancer Biology and Drug Discovery, China Medical University, Taichung 404, Taiwan
[4] Department of Biotechnology, Asia University, Taichung 413, Taiwan.

Abstract

The proviral insertion site in moloney murine leukemia virus (PIM) proteins family, including PIM1, PIM2, PIM3, are serine/threonine kinases and promote cell growth, proliferation and drug resistance. PIM1 is overexpressed in many cancer cells and plays critical role in tumorigenesis. Overexpression of Pim-1 has been found to be associated with the increases in protein levels of RTKs, HER3, EPHA2, HER2, and EGFR in prostate cancer cells. Silence of Pim-1 is also accompanied with downergulation of EGFR family. However, it remains unclear whether the kinase activity of Pim-1 is involved in the gene regulation of EGFR family. In this study, treatments with Pim inhibitors significantly reduced the viability of various breast cancer cell lines. Among these cell lines, HER2-positve breast cancer cells were more sensitive to these inhibitors. Inhibition of Pim kinase activity also reduced their migration and invasion activities. Moreover, Pim inhibitors suppressed the viability of acquired lapatinib-resistant HER2-breast cancer cells. Mechanistically, Pim inhibitors reduced the EGFR, HER2, and HER3 both at protein and mRNA levels, and induced apoptosis. These results suggest that Pim-1, relying on its kinase activity, may regulate EGFR family expression at transcriptional level. Our findings also suggests that downregulation of EGFR family by targeting Pim-1 may be a potential therapeutic strategy for HER2-positive breast cancer patients and for circumventing lapatinib resistance. However, identification of the more detail molecular mechanisms awaits further studies.

Results



b.

SGI-1776		
cells	IC50	HER2
SkBr3	3.0733	1.5109
sl/LR6	9.1317	1.1766
sk/LR9	11.0193	1.0393
MCF7	10.6003	0.1269
HER18	7.4117	1.3122
T47D	7.8585	0.0632
T47D Vector	8.6675	0.0032
T47D WT	12.1029	0.1869
BT474	5.7120	1.0470
MDA-MB 231	3.4861	0.0159
HBL-100	6.6468	0.0002

SGI-1776		
cells	IC50	HER3
SkBr3	3.07	1.23
sl/LR6	9.13	0.24
sk/LR9	11.02	0.31
MCF7	10.60	0.23
HER18	7.41	0.19
T47D	7.86	0.91
T47D Vector	8.67	0.69
T47D WT	12.10	0.27
BT474	5.71	0.67
MDA-MB 231	3.49	0.13
HBL-100	6.65	0.18

SMI-4a		
cells	IC50	HER2
SkBr3	14.5346	1.5109
sl/LR6	32.2469	1.1766
sk/LR9	13.4922	1.0393
MCF7	78.0103	0.1269
HER18	18.8149	1.3122
T47D	238.2131	0.0632
T47D Vector	145.7469	0.0032
T47D WT	19.1915	0.1869
BT474	36.0413	1.0470
MDA-MB 231	618.5906	0.0159
HBL-100	100.0110	0.0002

SMI-4a		
cells	IC50	HER3
SkBr3	14.53	1.23
sl/LR6	32.25	0.24
sk/LR9	13.49	0.31
MCF7	78.01	0.23
HER18	18.81	0.19
T47D	238.21	0.91
T47D Vector	145.75	0.69
T47D WT	19.19	0.27
BT474	36.04	0.67
MDA-MB 231	618.59	0.13
HBL-100	100.01	0.18

c.

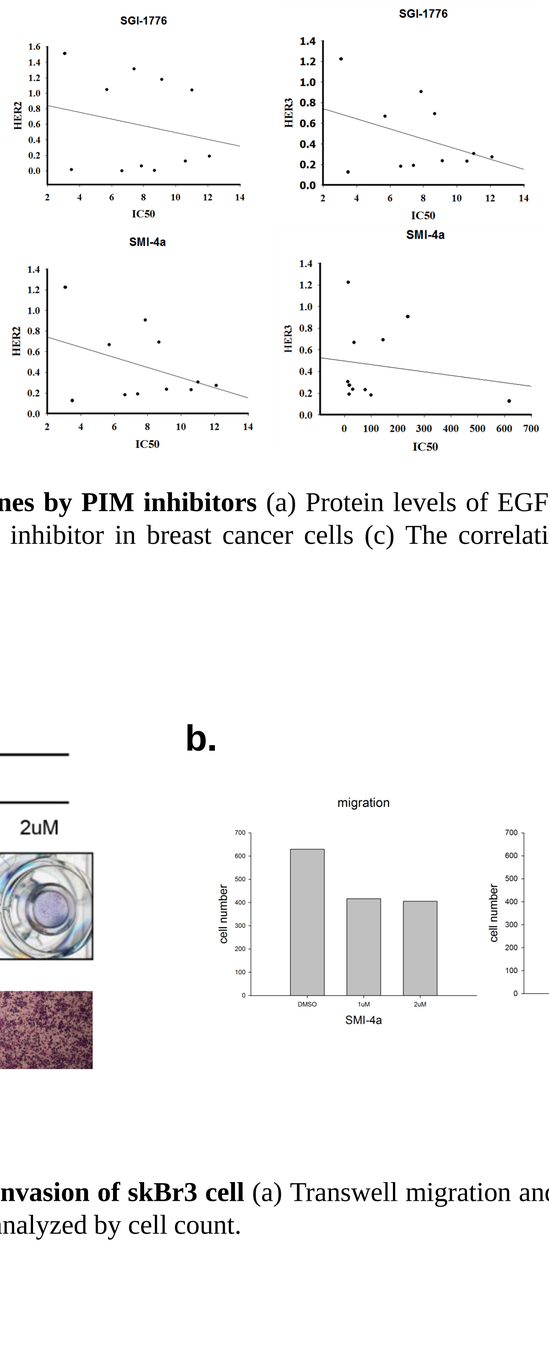


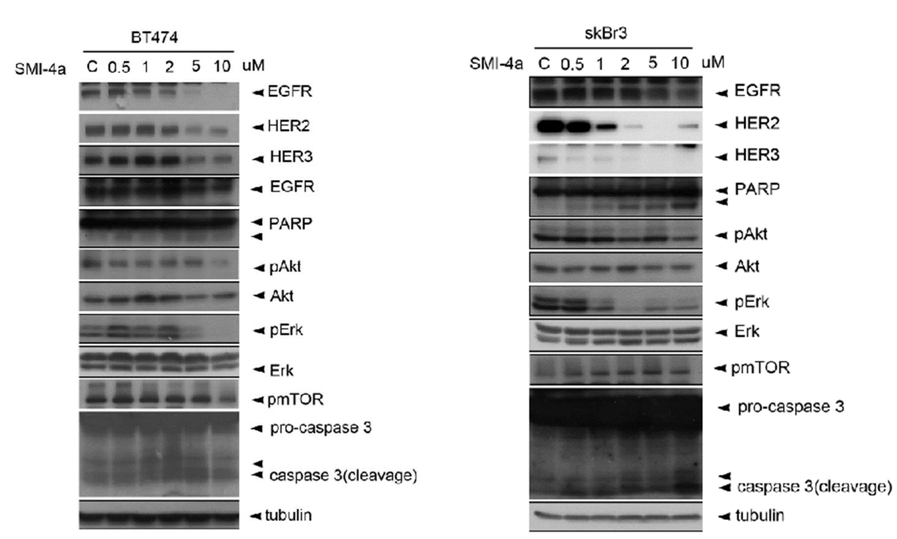
Fig.1 Viability inhibition of breast cancer cell lines by PIM inhibitors (a) Protein levels of EGFR family in breast cancer cells (b) Cell viability assay of Pim inhibitor in breast cancer cells (c) The correlation between EGFR family and cell viability of Pim inhibitors.

a.

b.

Fig.2 The Pim inhibitors reduce migration and invasion of skBr3 cell (a) Transwell migration and invasion assays of skBr3 cells treated with SMI-4a (b) and analyzed by cell count.

a.



b.

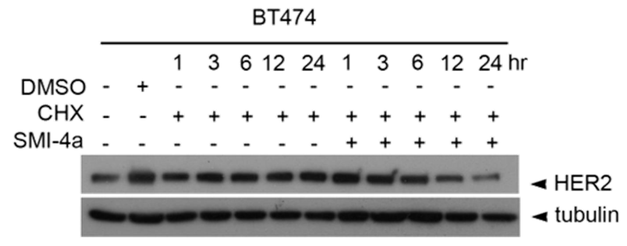


Fig.3 The Pim inhibitor reduce EGFR family expression in BT474 cell (a) Dose-dependent of Pim inhibitor in BT474 and skBr3 cells , cells were treated with SMI-4a 48hr. (b) that were treated for varying periods of time with cycloheximide (CHX).

a.

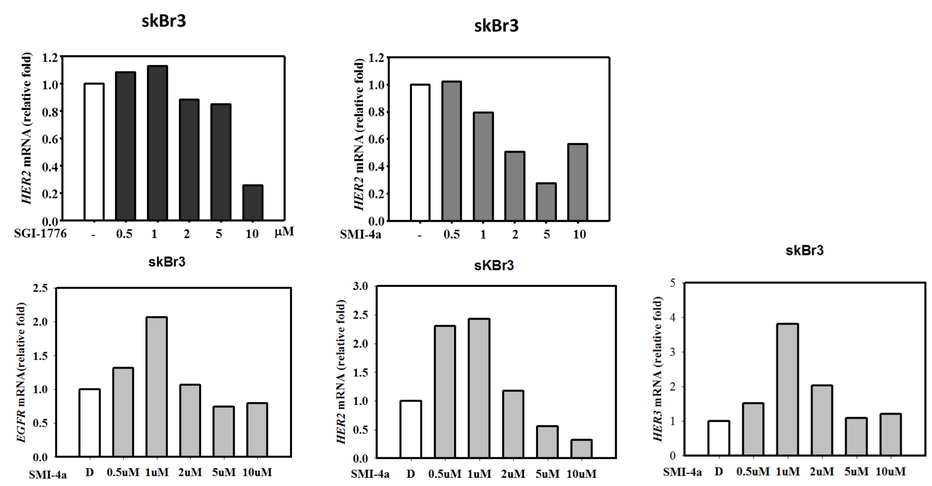


Fig.4 The Pim inhibitor reduce EGFR family mRNA expression in HER2 high breast cancer cells.(a) The mRNA levels of EGFR ,HER2 and HER3 were analyzed by real-time RT-PCR in skBr3 cells. Cell treated Pim inhibitor 48hr.

a.

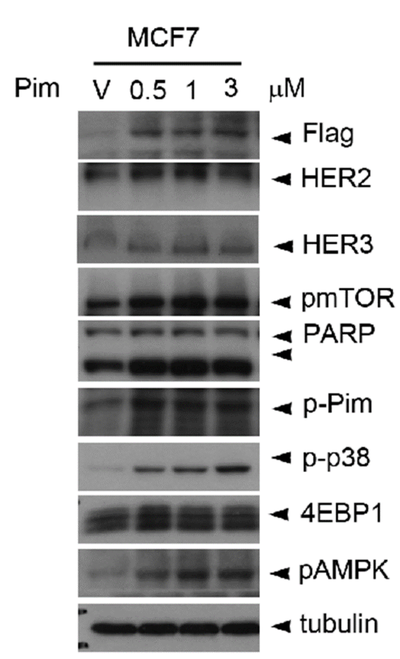


Fig.5 HER family were up-regulated through overexpression of Pim (a) MCF7 cells were transfected with a Pim-1, an empty vector, or a Pim-1– expressing plasmid for 24 hours.

Conclusions

- Treatments with Pim inhibitors significantly reduced the viability of various breast cancer cell lines.
- HER2-positve breast cancer cells were more sensitive to these Pim inhibitors.
- Inhibition of Pim kinase activity also reduced their migration and invasion activities.
- Pim inhibitors suppressed the viability of acquired lapatinib-resistant HER2-breast cancer cells.
- Pim inhibitors induced apoptosis.