C8

基礎組-從業

塞來昔布在大腸直腸癌中調控c-Met活性之機制探討

The mechanism underlying celecoxib-mediated regulation of c-Met activity in CRC cells

Kalu, Franklin Chikodi Udo[1], Pei-Hsuan Chien (簡佩萱)[2], Yuen-Ming Lin (林岳民)[3], Yun-Ju Chen (陳韻如)*[,1,2]

[1]School of Medicine for International Students, I-Shou University, Kaohsiung, Taiwan (義守大學學士後醫學系外國學生專班)

[2]Department of Medical Research, E-Da Hospital, Kaohsiung, Taiwan (義大醫院醫學研究部)

[3]Division of Colorectal Surgery, Department of Surgery, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan (高雄長庚醫院大腸直腸外科)

Abstract

Prevention of cancer relapse is now a challenge for cancer treatment. Several lines of evidence demonstrate that cancer stemness inhibition acts as a predictive factor for cancer relapse and is a potential therapeutic target. Our previous study indicates that celecoxib owns the most potent inhibitory activity against cancer stemness property of colorectal cancer (CRC) cells among a variety of non-steroidal anti-inflammatory drugs (NSAIDs). Analysis of underlying mechanism reveals that celecoxib inhibits both cyclooxygenase-2 (COX-2) activity and c-Met activity. Furthermore, c-Met is a critical factor for the cancer stemness property of CRC cells. In this study, we further investigated the mechanism underlying celecoxib-mediated inhibition of c-Met activity in CRC cells.

預防癌症復發是目前癌症治療的一大挑戰，很多證據已證實癌幹性抑制與否可作為癌症復發的預測因子及作為治療的標的。我們先前研究指出celecoxib是眾多非類固醇抗發炎藥中具有最強的大腸直腸癌幹性抑制作用，分析其中的機制，發現這是因為celecoxib可同時抑制COX-2及c-Met所致。此外，c-Met也是大腸直腸癌幹性調控的一個重要因子。在目前研究中，我們進一步探討celecoxib在大腸直腸癌細胞中如何抑制c-Met活性。

Methodology

1. ***In vitro kinase assay.*** In brief, the purified kinase domain of c-Met was incubated with commercial PTK substrates, Poly (Glu:Tyr, 4:1) in the presence of ATP with indicated treatment, including celecoxib, crizotinib and PHA665752 at 30° for 1 hour. Tyrosine phosphorylation was detected by using anti-phospho-tyrosine antibody.
2. ***Spheroid formation assay.*** CRC cells were trypsinized and washed with 1 X PBS twice, followed by last washing with spheroid-inducing medium. Then, cells previously subjected to transfection and with celecoxib treatment were seeded in a density of $5x10^4$ cells/ml in an ultra-low attached plate with spheroid-inducing medium. Cells were sieved with filter and refreshed with spheroid-inducing medium every week, allowing the formation of spheroid. Seven days later, the formation of spheroid was determined under microscope.

Results

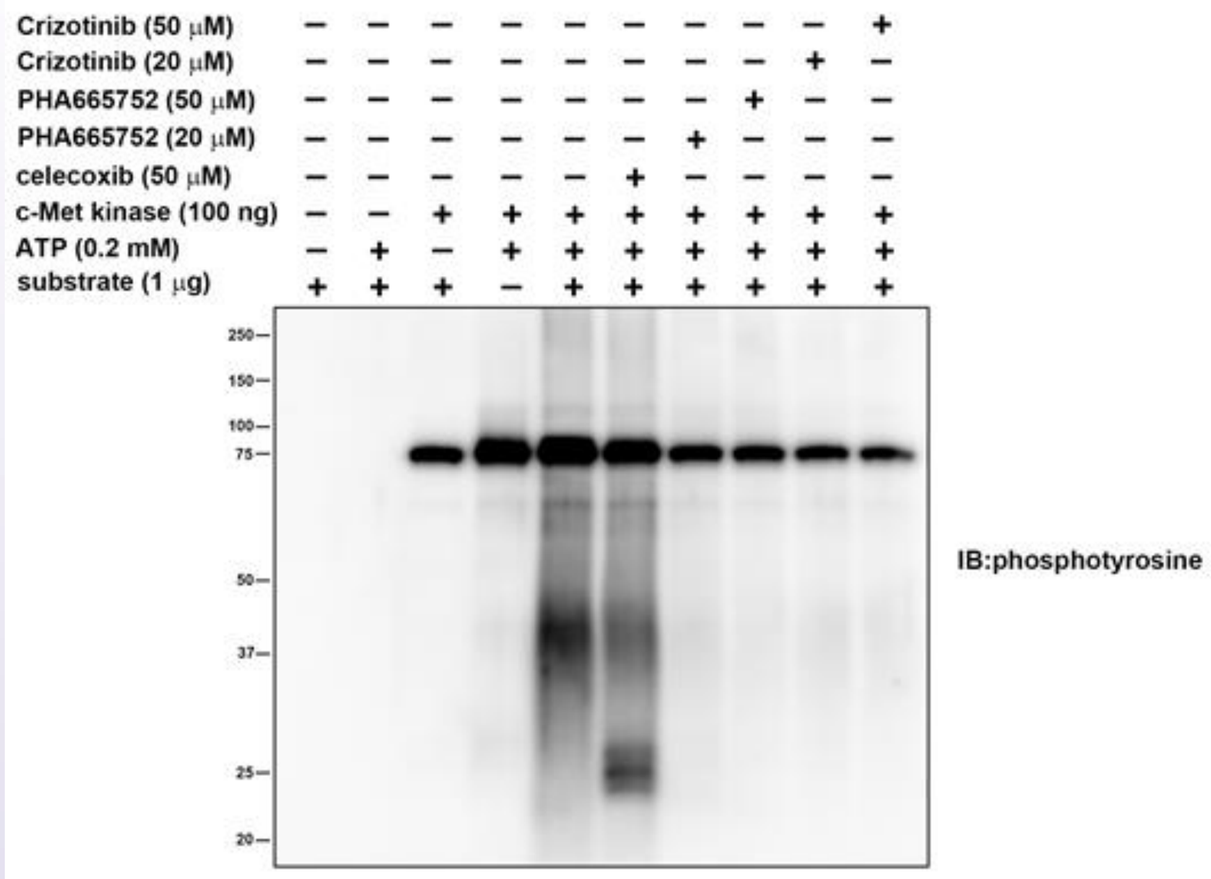

Figure 1. Celecoxib regulated c-Met activity indirectly.

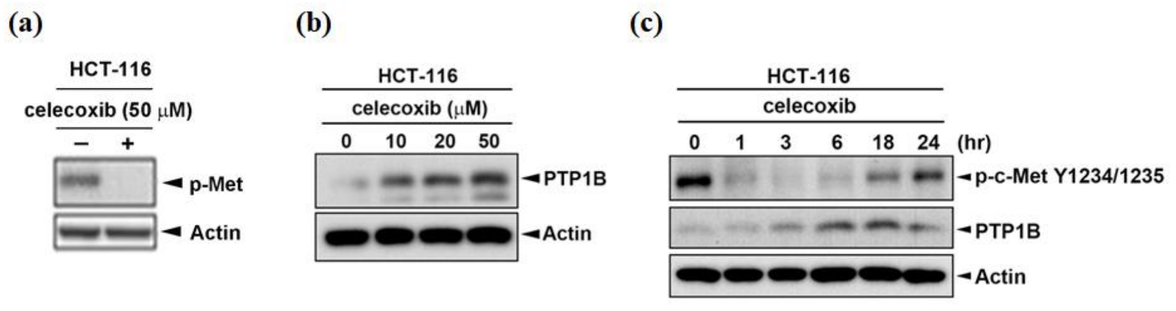

Figure 2. PTP1B expression was increased by celecoxib in CRC cells.

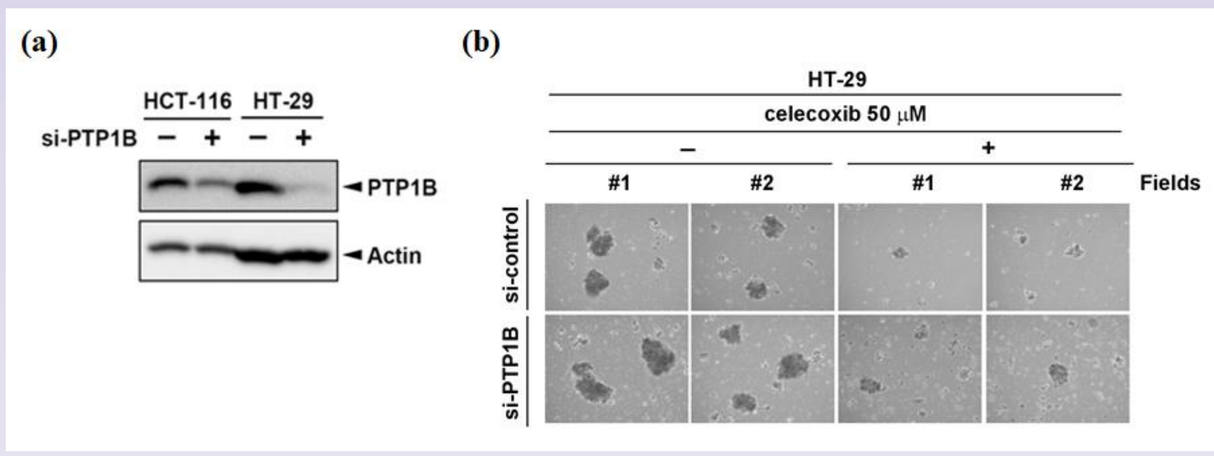

Figure 3. PTP1B was involved in the regulation of CRC stemness property by celecoxib.

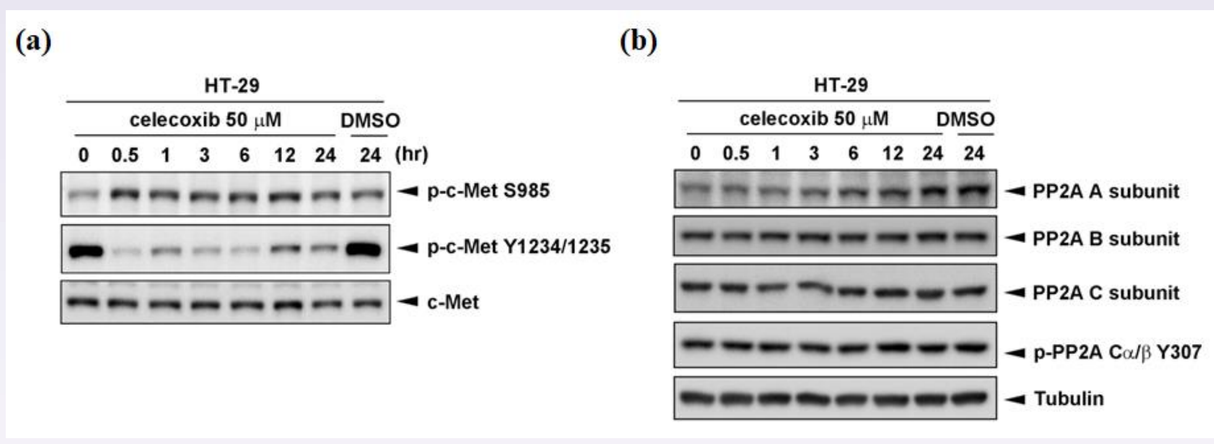

Figure 4. Serine 985 phosphorylation of c-Met was involved in the celecoxib-mediated regulation of its activity.

Summary

1. The results showed that c-Met kinase activity was only slightly inhibited by celecoxib whereas totally blocked by well-known classical c-Met kinase inhibitors as shown by *in vitro* kinase assay, suggesting that celecoxib did not directly bind to c-Met to inhibit its kinase activity.
2. Tyrosine 1234/1235 phosphorylation of c-Met, an indicator of activation, was attenuated in celecoxib-treated CRC cells. Protein tyrosine phosphatase 1B (PTP1B), one of known negative regulators of c-Met, was increased by celecoxib in a dose-dependent manner. Furthermore, celecoxib exerted both time-dependent inhibition of tyrosine 1234/1235 phosphorylation of c-Met and increase of PTP1B protein expression.
3. Next, we examined the involvement of PTP1B in the regulation of CRC stemness property by celecoxib and silence of PTP1B expression by siRNA approach was applied. As shown in results, PTP1B expression was apparently depleted by siRNA in two CRC cells. We further examined the effects of PTP1B silence on CRC stemness property and found that spheroid formation was slightly increased in CRC cells without PTP1B expression. The mechanism underlying involvement of PTP1B in the regulation of CRC stemness property by celecoxib will be continuedly explored.
4. On the other hand, we investigated the involvement of other known negative regulators of c-Met activity in this regulation. It is known that protein phosphatase 2A (PP2A), a serine/threonine phosphatase, is reported to mediate serine 985 dephosphorylation of c-Met and in turn to enhance tyrosine 1234/1235 phosphorylation of c-Met. The results showed that serine 985 phosphorylation of c-Met was obviously increased at 30 minutes of celecoxib treatment and lasted for 12 hours, which negatively corresponded to the pattern of tyrosine 1234/1235 phosphorylation of c-Met. However, the expression and phosphorylation of protein phosphatase 2A were not affected by celecoxib treatment in CRC cells, suggesting the involvement of positive regulator of serine 985 phosphorylation of c-Met. The roles of serine 985 phosphorylation of c-Met in this regulation awaits further investigation in the future.

Funding

This work was supported by grants from E-Da Hospital (EDAHS108005).