Intracellular calcium influx mediates invasiveness of colon cancer cell via destabilization of focal adhesion kinase

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Carcinogenic induction in a colon occurs through a sequence of events leading to metastasis that involved various oncogenic proteins. Focal adhesion kinase (FAK) regulates metastatic adhesion of carcinoma cells, and it has recognized as a potential therapeutic target to metastatic colon cancer. However, calcium (Ca\textsuperscript{2+}) dependent calpain-FAK pathway to clear up the mechanism of motility has not been understood. Recently, Ca\textsuperscript{2+} bound lactate was used to induce intracellular Ca\textsuperscript{2+} (iCa\textsuperscript{2+}) influx into colorectal cancer (CRC) cells, and we confirmed that iCa\textsuperscript{2+} influx mediated FAK destabilization and CRC cell motility. Calpeptin, a calpain inhibitor, restored the effect of iCa\textsuperscript{2+} influx on the CRC cells. We herein discuss the phenomenon of an increase in the CRC cell motility that focused on the iCa\textsuperscript{2+} influx-induced FAK cleavage via calpain.

Keywords: Colon cancer cell; calcium influx; focal adhesion kinase; calpain; invasion


The intracellular calcium (iCa\textsuperscript{2+}) ion is an important signaling molecule that modulates numerous cellular processes in cancer [1]. Ca\textsuperscript{2+} permeable channels such as stored-operated calcium channels, transient receptor potential channels and the calcium release activated channel protein 1 are involved in the iCa\textsuperscript{2+} homeostasis. Remodeling or deregulation of iCa\textsuperscript{2+} homeostasis in cancer cells causes changes in cancer progression [2].

Focal adhesion kinase (FAK) plays a critical role in colon cancer progression, and it involved in cancer cell motility [3]. Calpain is a family of intracellular cysteine protease whose potentially induces cleavage of focal adhesion proteins depend on an increase in the iCa\textsuperscript{2+} level. Calpain-mediated FAK degradation is critical in cancer cell motility [4]. The scaffolding function of FAK brings calpain into a focal adhesion complex, resulting in phosphorylation of calpain [5]. This phosphorylation leads to the activation of calpain, and then it leading to adhesion complex turnover and cell migration. Therefore, metastatic tumors contain higher level of calpain than non-metastatic tumors comparatively [6].

However, the impact of Ca\textsuperscript{2+} supplementation has not been clearly understood on cancer metastasis. Based upon the results on direct exposure of lactate-bound Ca\textsuperscript{2+} in colorectal cancer (CRC) cells, we highlight the increased motility of CRC cells that focused on the calpain-FAK pathway to understand underlying molecular mechanisms of human CRC cell motility by iCa\textsuperscript{2+} [7]. It is well-known that lactate is highly utilized in the oxygenated cancer cells. Utilization of the lactate-bound Ca\textsuperscript{2+} originated from this cancer’s characteristic capacity to induce an increase in the Ca\textsuperscript{2+} influx via lactate channel [8].

Therefore, we investigated the impact of supplemental Ca\textsuperscript{2+} on the molecular mechanisms of metastatic CRC. Firstly, we analyzed the level of iCa\textsuperscript{2+} in the CRC cells (HCT-116 and HT-29) after lactate-bound Ca\textsuperscript{2+} treatment to confirm the
continuous Ca\(^{2+}\) influx. It showed a remarkable increase in the Ca\(^{2+}\) influx by the treatment of lactate-bound Ca\(^{2+}\). The lactate intake into oxygenated tumor cells is mediated via monocarboxylate transporters 1 \([9]\). It appears that lactate-bound Ca\(^{2+}\) is being transported into the cytoplasm, thus, increasing iCa\(^{2+}\) is coincidently observed \([7]\). However, the specific transport mechanism did not identify.

In the previous studies, the carcinogenesis of human CRC is related to the FAK destabilization \([3, 10, 11]\). By treatment of lactate-bound Ca\(^{2+}\), the full length of FAK is cleaved into a 90 kDa N-FAK protein (FERM domain). pFAK was also cleaved in to its p-N-FAK after the treatment of lactate-bound Ca\(^{2+}\) \([7]\). These results indicated that an increase in the iCa\(^{2+}\) destabilized the FAK and pFAK that caused by cleavage in the protein level of FAK and pFAK.

Since lactate-bound Ca\(^{2+}\) increased Ca\(^{2+}\) influx and cleaved FAK and pFAK, we determined the effects of calpain in the CRC cells. Calpain is involved in several key aspects of cancer cell adhesion, migration, and metastasis, and it is well known that FAK is cleaved by a Ca\(^{2+}\)-dependent protease calpain in motile cells including CRC cells \([5, 12]\). To confirm the calpain activation by Ca\(^{2+}\) influx, we used a rho kinase activator and an inhibitor of calpain that is known as calpeptin \([13]\). Calpeptin reversed the effects of lactate-bound Ca\(^{2+}\) on FAK and pFAK cleavage in the CRC cells \([7]\). Therefore, recent studies indicated that iCa\(^{2+}\)-contributed calpain activation is an important to CRC cell motility through its capacity to regulate focal adhesion dynamics.

Nuclear fraction of lactate-bound Ca\(^{2+}\)-treated CRC cells also showed an increase in the level of FAK and cleaved FAK. Translocation of FAK to nuclear can promote cell survival, it is well known that cleaved FAK mediated a degradation of p53 through nuclear translocation of cleaved N-FAK \([14, 15]\). In accordance with our results, the confocal microscopy of control cells showed DAPI stained nuclei without FAK. However, the lactate-bound Ca\(^{2+}\)-treated cells show translocation of N-FAK into DAPI stained nuclei. These results clearly indicate that cleaved N-FAK translocated into the nucleus of the CRC cells. And, the increased influx of Ca\(^{2+}\) can cause proteosomal degradation.

Figure 1. Schematic of a Ca\(^{2+}\) influx dependent FAK pathway in the CRC cell. Increased Ca\(^{2+}\) influx by calcium lactate induced calpain activity. Cleaved FAK (N-FAK or FERM-FAK) translocated into nucleus of the CRC cells, and P53 oncosuppressor gene is inactivated by MDM2 and the cleaved FAK. That is, Ca\(^{2+}\) influx dependent cleaved FAK is related to an increase in the CRC cell survival and motility.
of p53 through nuclear translocation of FERM-FAK and mdm2-mediated ubiquitination\[7,15].

We finally confirmed that the effects of lactate-bound Ca:\(^{2+}\) on these cellular properties in the CRC cells. Therefore, invasiveness of the CRC cells was investigated using the wound healing assay. Results demonstrated that the treatment of lactate-bound Ca:\(^{2+}\) increased the motility of the CRC cells. Single treatment of calpeptin did not give any effect to the motility of the CRC cells. Interestingly, it was shown that combination application of lactate-bound Ca:\(^{2+}\) with calpeptin inhibited the CRC cell motility\[7\]. To delineate the effect of Ca:\(^{2+}\) influx-mediated FAK degradation through calpain activity in a condition of the increased CRC cell motility, the invasiveness of the CRC cells was also confirmed after pFAK inhibitor (TAE226) treatment. These results clear up the essential point that iCa:\(^{2+}\)-mediated pFAK cleavage is critical in the CRC cell motility\[3,7,15\].

In conclusion, the present study elucidates that Ca:\(^{2+}\) influx via lactate-bound Ca:\(^{2+}\) increased motility of the CRC cells by calpain activity through destabilizations of the FAK proteins (Figure 1). The one feature of this study has kept in mind that the effect of Ca:\(^{2+}\) influx was investigated in the early time after treatment of lactate-bound Ca:\(^{2+}\). Because, the level of iCa:\(^{2+}\) concentration reached to maximum between 100 and 480 seconds, and then the level was plateaued \[7\]. The importance of lactate-bound Ca:\(^{2+}\) in a modulation of colon cancer cell physiology is not limited to basic studies but also have an impact on clinical nutritional outcome. The increased iCa:\(^{2+}\) plays various roles in cancer progression controversially, including increasing apoptosis or inhibiting proliferation in the colon \[16,17\]. Furthermore, Ca:\(^{2+}\) binding to bile salts, increased in high fat diets, have been associated with colonic carcinogenesis \[18\]. Lactate-bound Ca:\(^{2+}\)-induced FAK and pFAK cleavage by calpain and enhanced cell motility of the CRC cells create an assumption that metastatic colon cancer patients need more careful when considering dietary supplementation of Ca:\(^{2+}\). Because extracellular calcium can be ionized with lactate, it leads to Ca:\(^{2+}\) influx causing increased the CRC cell motility.

Conflicting interests

The authors have declared that no competing interests exist.

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Author Contributions

Hwan Mook Kim, Keun-Yeong Jeong, Pasupathi Sundaramoorthy, and Jae Jun Sim designed the outline. Hwan Mook Kim and Keun-Yeong Jeong reviewed the previous results. Keun-Yeong Jeong and Pasupathi Sundaramoorthy wrote the paper.

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