I. INTRODUCTION

Osteosarcoma (OS) is a bone cancer that happens due to the formation of an immature osteoid matrix produced from primitive bone-forming (osteoid-generating) mesenchymal cells (Sadykova et al., 2020; Prater & McKeon, 2022). OS is known to be the 7th most common cancer in children which also happens to be the most common primary bone cancer in childhood with an annual incidence of 5.2 per million people in children aged 0 to 19 years old (Ward et al., 2014; Sadykova et al., 2020). OS is known for its high proliferation ability through the activation of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (Dong et al., 2014). This high proliferation ability is proven by OS's ability to proliferate to surrounding organs as well as to distant organs such as the lungs (Gazouli et al., 2021). However, several other mechanisms could play a role in this high proliferation ability of OS that is still undergoing research.

Cytidine triphosphate synthase (CTPS) is one of the crucial enzymes that participate in structural components of DNA and RNA in the de novo synthesis (Chang et al., 2017; Chakraborty et al., 2020). CTPS1 forms filamentous structures across species and the formation requires post-translational changes such as ubiquitination and methylation because it is a dynamic process (Chakraborty et al., 2020). Previous studies on many cancers including breast cancer, colon cancer, and ovarian cancer have reported the formation of filamentous structures under stress conditions. It is also known that both CTPS1 and NME1 lay near the keratin network and are able to interact with each other under stress conditions, such as glutamine deprivation.

Glutamine is one of the most abundant amino acids in plasma which is utilized by cancer cells as a source of energy in their growth and proliferation while the pro-oncogenes are activated (Lin et al., 2018). Following its entry into cells, glutamine serves as a precursor for producing several amino acids, proteins, nucleotides, and other compounds essential to biological processes such as the TCA cycle.

YAP or YAP1, a transcription co-activator in the hippo pathway, directly raises the expression and activity of glutamine synthetase, increasing glutamine levels in the steady state and improving nitrogen's relative isotopic enrichment during de novo purine and pyrimidine production (Cox et al., 2016). Therefore, glutamine deprivation will lead to a disruption in the growth and proliferation of the cancer cells. Interestingly, glutamine deprivation is related to a filamentous structure in cancer cells. Besides that, the downstream genes of the hippo pathway such as CCN1 or CYR61 and CCN2 or CTGF participate in cell adhesion and migration as CCN are part of the extracellular matrix (ECM) (Kim et al., 2018).

Therefore, this study aims to see how CTPS filament formed in U2OS cells under glutamine deprivation, as well as to examine whether CTPS1 and its protein complex under glutamine deprivation affect the YAP downstream genes in U2OS cells. It is also hypothesized that filament can form in U2OS cells under glutamine deprivation and YAP downstream gene will increase in U2OS cells under glutamine deprivation.