CHAPTER 1

INTRODUCTION

1.1 Problem Background

Cancer is a complex disease. It starts with genetic mutation that accumulates over time; which leads to rapid, uncontrollable growth of cells. This cell mass has the ability to invade nearby tissues or organs, even the distant ones, disrupting their normal function and causing damages (Luo, Solimini, & Elledge, 2009). Eventually, cancer cell takes over by using body's own biological mechanism against itself, killing the host.

Once regarded as a challenge in the west, now it has been the leading cause of death in the world (International Agency for Research on Cancer, 2014). The incidence of this threat may be relatively lower in India and Indonesia, but as low-resource countries, late-stage diagnosis of the disease results in higher mortality rate and poor clinical outcomes (World Health Organization, 2014). On the other hand, conventional therapies may be helpful to an extent, but not without side effects that increase the disease burden (International Agency for Research on Cancer, 2014), with a chance of relapse or developing secondary cancer. The search for the means to combat cancer is not over.

Over the years, molecules that are involved in cancer pathophysiology have been studied to develop treatments. This includes matrix metalloproteinases (MMPs), enzymes that degrade proteins in the extracellular matrix (ECM). Its function makes MMPs crucial in several processes, such as tissue remodeling and intercellular communication (Kessenbrock, Plaks, & Werb, 2010). However, if deregulated, MMPs can promote cancer cells to grow and metastasize (Jabłońska-Trypuć, Matejczyk, & Rosochacki, 2016). Decreased prognosis is also correlated with the elevated MMPs level in various cancer types - making it an attractive drug target for cancer (Vihinen & Kähäri, 2002).

Unfortunately, there are still no MMP inhibitors effective enough to enter the market. Synthetic MMP inhibitors showed promising results in the cell culture experiments, but they had been failing in the clinical trials (Gialeli, Theocharis, & Karamanos, 2011). This reflects the gap between *in vitro* and *in vivo* models. It is imperative that the initial observation gives more representative data to mitigate the lengthy and costly process of drug discovery.

In *in vitro* cancer research, 2D monolayer culture has been very useful to test potential therapeutic agents. Its advantages include simplicity, cellular uniformity, and practicality, making it easy to control in the laboratory environment (Fennema, Rivron, Rouwkema, van Blitterswijk, & De Boer, 2013). However, it is far from resembling the actual tumor microenvironment. This means that the results are most likely unreliable and non-predictive to the *in vivo* model or the human body.

In body tissues, solid cancer cells grow in contact with each other, with cellular heterogeneity, and in hypoxic condition. Multicellular tumor spheroid (MCTS) culture technique can replicate these conditions in a simple and reproducible manner (LaBarbera, Reid, & Yoo, 2012). However, not every cell line can produce spheroids, and each may require different conditioning (Weiswald, Bellet, & Dangles-Marie, 2015). Nevertheless, even though the 3D culture technique is not a perfect answer, it has been proven to be the bridge between 2D culture experiments with *in vivo* models. Most importantly, it is able to produce similar barrier to drug penetration and more accurate response to determine drug effectiveness before proceeding in drug screening (Minchinton & Tannock, 2006).

1.2 Problem Formulation

For this undergraduate thesis project, MMP activity is observed in the spheroid of HT-1080, a fibrosarcoma cell line. Fibrosarcoma is a very rare, metastatic soft tissue sarcoma of the fibroblasts (Folpe, 2014). These cells produce a considerable amount of MMP-9 and -2, gelatinases that are most commonly correlated with cancer metastasis – making it suitable for MMP studies (Roomi,

Kalinovsky, Monterrey, Rath, & Niedzwiecki, 2013). This had also been observed in the previous experiments, but in the form of 2D culture (Nambiar et al., 2016).

These observations beget the following interests: (1) the best method to form HT-1080 spheroids, (2) the effect of MMP on those spheroids, and (3) the difference of MMP activity between the 2D and 3D culture of HT-1080. Overall, this project is done under the assumption that HT-1080 cell line as spheroids in 3D cell culture has different response to and activity of MMP compared to 2D cell culture, which can be determined in the laboratory environment to provide more predictive results of *in vitro* experiments in drug discovery.

1.3 Research Objectives

The 4 main objectives in these experiments are:

1. To establish the most suitable condition for HT-1080 to form spheroids in this experiment. Different concentrations of four different substrates are tested: agar, agarose, collagen, and gelatin; by using 2 different techniques to form spheroids: liquid overlay and embedded.
2. To decide the most suitable media for HT-1080 spheroid cell culture by observing the difference of using complete medium (with 10% FBS) and serum-free media (SFM) on the formation and growth of spheroids and MMP activity.
3. To determine the effect of MMP overexpression during and after the spheroid formation by using phorbol 12-myristate 13-acetate (PMA) as an inducer of an MMP-rich environment.
4. To test the established condition of HT-1080 spheroids using natural extracts. Leaf and stem extracts of *Simarouba glauca* are added to the spheroids to observe the difference of MMP inhibition with the previous 2D cell culture experiments of HT-1080.

1.4 Research Scope

The focuses of this undergraduate thesis project are as follow:

1. The type of cancer is limited to solid tumor, particularly HT-1080 fibrosarcoma cell line, chosen because of its ability to produce significantly detectable amount of MMPs.
2. The types of MMP observed are MMP-2 and -9.
3. The 3D cell culture techniques tested are for multicellular tumor spheroid (MCTS) production with liquid overlay and embedded technique.
4. The substrates tested for the coating material are agar, agarose, collagen, and gelatin.
5. The media tested is DMEM with 1% mixture of penicillin, streptomycin, and amphotericin; with or without 10% FBS.
6. The MMP inducer chosen is PMA, the activator of MMP-9.
7. The natural extract is from the leaf and stem of *Simarouba glauca*, of which the preliminary data exists as a comparison, to test MMP-2 and -9 detection from HT-1080 spheroid model.
8. The analysis involves only the use of bright field microscopy and gelatin zymography with their respective software and Microsoft Excel.