CHAPTER 1

INTRODUCTION

1.1. Introduction

The Cell-matrix adhesion can be categorized as primary structural support in the normal cellular migration process. A Proper cell-adhesion signaling is a crucial mechanism as it determines various cellular processes such as cell polarity, proliferation, apoptosis, differentiation, and migration (Berrier & Yamada, 2007; Desgrosellier & Cheresh, 2010; Maître & Heisenberg, 2013). The cell initiates its migration process by reconstructing its cytoskeleton structure, thus creating a 'tentacle-like' organ called a protrusion body. This distinct structure enables the cell to reach its surrounding area, establishing a focal contact with extracellular matrix (ECM), and start dragging its whole body towards the protrusion body growth direction. This protrusion body termed as filopodia consist of F-actin cytoskeleton structure that allows its tip to establish a focal contact with ECM by creating a cluster of protein in between the cell membrane and ECM called focal adhesion (Figure 1.1) (Krakhmal et al., 2015; Jacquemet, Hamidi, & Ivaska, 2015; Wu C. 2007).

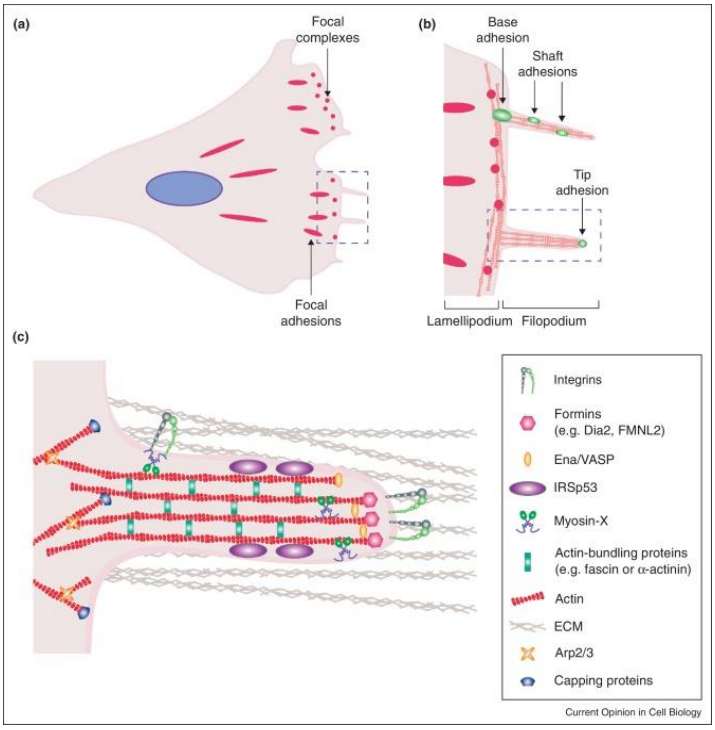

Figure 1.1. Filopodia formation in a cancer cell (a and b) and integrin as adhesion protein needed to connect actin cytoskeleton with ECM (c) (Jacquemet, Hamidi, & Ivaska, 2015)

The Focal adhesion works to create a signal by sensing the surrounding tissue condition; for example, extracellular matrix rigidity relays the signal to the nucleus thus triggering biological responses (Vogel & Sheetz, 2006; Trubelja & Bao, 2018). It consists of integrins as the main actor and different focal adhesion complexes to support integrin-actin connection such as vinculin, talin, formin, focal adhesion kinase (FAK), zyxin, and paxillin (Kim & Wirtz, 2013). The earliest focal adhesion proteins recruited and bind to integrin are paxillin and talin (Lawson & Schlaepfer, 2012). Paxillin serves as a protein docking site that allows different signaling molecules such as tyrosine kinases, serine/threonine kinases, cytoskeletal protein, and adaptor protein to bind. It also recruits additional enzyme needed to build a focal adhesion (Schaller, 2001).

Along with paxillin, talin serves as a mechanosensor protein that directly connects integrin and actin cytoskeleton. Talin transmit force from outside towards the inside of the cell resulting in the formation of actin contractility. On the other hand, talin can also transmit forces from inside towards outside of the cell in the form of matrix stretching (Klapholz & Brown, 2017; Das, Ithychanda, Qin, & Plow, 2014; Yao, 2016). In order to perform those functions, talinconsists of head and rod domain in which both of the domains are constructed from several subdomains (Figure 2.2A). Each of the subdomains consists of various protein binding sites in which each of them bears distinctive function that exposes talin to multiple intracellular signaling.

Within talin head domain, there is one distinct subdomain called F0 subdomain that only present in some proteins that are able to activate integrin such as, talin and kindlin (Ceccarelli et al., 2006; Goult et al., 2009; Han, Nunomura, Takakuwa, Mohandas, & Jap, 2000; Liu, Zhu, Ye, & Zhang, 2012). However, this specific subdomain does not present in a similar head domain structure within other protein that does not have the ability to activate integrin, such as Focal Adhesion Kinase (FAK) (Franchini, Clemente, & Marin, 2009; Guan, 1997).Current studies manage to indicate the presence of F0 in both talin and kindlin intensifying and supporting the integrin activation process (Bos et al., 2003; Goult et al., 2009). However,the signaling pathway on how F0 subdomain support the integrin activation and how F0 subdomain presence affecting focal adhesion are still unknown.

Other important subunits that enable talin to bear the essential function within focal adhesion are F3 subdomain and R11 subdomain (Figure 2.2B). Both of these subdomains are classified into the integrin binding site (IBS) which differentiated based on their function. F3 subdomain works primarily to activate integrin while the R11 subdomain works to stabilizing the integrin to actin connection (Moes et al., 2007). Aside from F3 subunit that is widely studied and the current studies that encompass each of the subunit protein binding interaction and their main function; the detail signaling pathway especially on how R11 independently perform its function and its independent importance in focal adhesion formation is still unknown.

All of the focal adhesion protein need to complement their function to prevent cell-matrix adhesion impairment that can affect the homeostasis and immune response stability, blood formation and function, wound healing mechanism, even cancer progression, and cancer metastasis (Chinthalapudi, Rangarajan, & Izard, 2018; Duperret & Ridky, 2013). Through this project, two of the protein binding site within talin such as F0 subunit in talin head and R11 in talin rod was assessed to study its support in focal adhesion formation. Specifically on R11, as it has a similar protein binding site with F3 subdomain, this project is focusing on investigating F3-independent R11 effect on focal adhesion formation.

1.2. Objective

- To investigate the effect of F3-independent R11 deletion in talin-rod towards focal adhesion formation
- To investigate the effect of F0 subdomain deletion in talin rod towards focal adhesion formation