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Drug Resistance Updates 8 (2005) 51–58
DRUG RESISTANCE Updates
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Fibroblast activation protein-α and dipeptidyl peptidase IV (CD26): Cell-surface proteases that activate cell signaling and are potential targets for cancer therapy

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Received 22 February 2005; received in revised form 7 March 2005; accepted 8 March 2005

Abstract

Fibroblast activation protein-α (FAP-α) and dipeptidyl peptidase IV (DPPIV) are serine proteases with post-prolyl peptidase activities that can modify tumor cell behavior. FAP-α and DPPIV can form heteromeric complexes with each other and may function coordinately to modulate the growth, differentiation, adhesion, and metastasis of tumor cells. This review is focused on FAP-α and summarizes a series of studies showing that elevated expression of FAP-α results in profound changes in growth and malignant behavior of tumor cells. Depending on the model system investigated, FAP-α expression causes dramatic promotion or suppression of tumor growth. In the case of tumor promotion, FAP-α expression can drive tumor growth by increasing angiogenesis and by decreasing the anti-tumor response of the immune system. In the case of tumor suppression, FAP-α can decrease tumorigenicity of mouse melanoma cells and restore contact inhibition and growth factor dependence even when it is catalytically inactive, implying that protein–protein interactions mediate these effects. Understanding how FAP-α activates cell signaling is critical to determining how FAP-α mediates growth promotion versus growth suppression in the different model systems and ultimately in human cancer patients. In particular, the roles of FAP-α protease activity and FAP-α complex formation with DPPIV and other surface molecules in activating cell signaling need to be elucidated since these represent potential targets for therapeutic intervention.

Keywords: Serine protease; Seprase; Metastasis; Cell proliferation; Angiogenesis

1. Introduction

The post-prolyl peptidases are a class of enzymes that are inducible, active on the cell surface or in extracellular fluids, and uniquely capable of cleaving the Pro-XAA bond. These enzymes have important roles in cancer (for reviews see (Chen and Kelly, 2003; Chen et al., 2003; Rosenblum and Kozarich, 2003; Busek et al., 2004). Through their enzymatic activities, the post-prolyl peptidases modify bioactive peptides and change cellular functions. This group of enzymes includes quiescent cell proline amino peptidase, prolyl carboxy-peptidase, prolyl endopeptidase, dipeptidyl peptidase 6, dipeptidyl peptidase 8, dipeptidyl peptidase 9, attractin, dipeptidyl peptidase II, and dipeptidyl peptidase IV-β (see Chen et al., 2003). But, the best studied of the post-prolyl peptidases is the cell surface serine protease, dipeptidyl peptidase IV (DPPIV or CD26). Recent studies have shown the importance of DPPIV in regulating tumor cell behavior and function (Bauvois, 2004). This review is focused on fibroblast activation protein-α (FAP-α, also known as "seprase"), which is another post-prolyl peptidase and cell surface serine protease that is closely related to DPPIV. FAP-α has important roles in tumor biology that are just beginning to be understood. Clues provided by the relatively large volume of work done on DPPIV have yielded important insights into the potential functions of FAP-α and examples of these are highlighted throughout the text. Indeed, FAP-α and DPPIV may function coordinately to regulate tumor cell behavior and both enzymes are

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doi:10.1016/j.drup.2005.03.002

appealing targets for therapies designed to eradicate cancer cells.

2. Aberrant expression of fibroblast activation protein-α in the tumor microenvironment

FAP-α is a cell-surface serine protease that was originally identified as an inducible antigen expressed on reactive stromal fibroblasts (Rettig et al., 1988,1993; Garin-Chesa et al., 1990). FAP-α was independently identified by Chen and coworkers as a 170-kDa membrane-associated gelatinase that is expressed by aggressive melanoma cell lines and transformed chicken embryo fibroblasts (Aoyama and Chen, 1990; Kelly et al., 1994; Monsky et al., 1994). These workers called this gelatinase "seprase" for surface expressed protease (Monsky et al., 1994). Subsequent molecular cloning of FAP-α and seprase revealed that they are the same cell-surface serine protease (Scanlan et al., 1994; Pineiro-Sanchez et al., 1997; Chen and Kelly, 2003; Chen et al., 2003). For clarity, the protease is referred to as FAP-α throughout this review.

FAP-α is not expressed by normal adult tissues; however, FAP-α expression is induced in activated fibroblasts responding to wounding and the reactive stroma responding to epithelial cancers and some sarcomas (Rettig et al., 1988,1993; Garin-Chesa et al., 1990). For example, FAP-α is induced in breast cancer. In fact, several groups have demonstrated FAP-α expression in the reactive stromal fibroblasts of human breast cancer and its absence of expression in normal breast tissue (Garin-Chesa et al., 1990; Rettig et al., 1993; Scanlan et al., 1994; Ariga et al., 2001). These findings were extended by work showing that in addition to reactive stromal fibroblasts, FAP-α is also expressed by infiltrating ductal carcinoma cells in breast cancer patients while it is not expressed by normal breast epithelia (Kelly et al., 1998). Initially, the finding of FAP-α expression in breast carcinoma cells was somewhat controversial, but subsequent reports investigating several different types of cancers confirmed that FAP-α expression is not restricted to stromal fibroblasts and is, in fact, expressed by at least some types of malignant cells of epithelial origin (Chen et al., 2003; Iwasa et al., 2003; Jin et al., 2003; Okada et al., 2003). Thus, FAP-α may have a critical role in shaping the microenvironment to promote tumor growth (Cheng and Weiner, 2003). Intense research is ongoing to determine the role of FAP-α in breast cancer and the mechanisms by which it mediates its functions.

3. FAP-α: a member of the post prolyl peptidase family of enzymes known to regulate cell behaviors

The post-prolyl peptidases are uniquely capable of cleaving the prolyl peptide bond Pro-XAA. This emerging group of prolyl oligopeptidases comprises a family with three main subdivisions as defined by sequence homology (Barrett and Rawlings, 1992; Sedo and Malik, 2001; Chen et al., 2003; Rosenblum and Kozarich, 2003). Prolyl endopeptidase is prototypical of the S9a family and acylaminoacyl peptidase of the S9c family. FAP-α and DPPIV (also called CD26) belong to the S9b peptidase family and are highly homologous. Indeed, FAP-α and DPPIV likely diverged after gene duplication (Irwin, 2002). FAP-α has an identical domain structure and 50% amino acid identity to DPPIV over the entire sequence, with almost 70% identity in the catalytic domain (Scanlan et al., 1994; Goldstein et al., 1997; Pineiro-Sanchez et al., 1997).

3.1. FAP-α structure

FAP-α is a type II transmembrane protein of 760 amino acids that is anchored in the plasma membrane by an uncleaved signal sequence of approximately 20 amino acids and has a short, amino terminal, cytoplasmic domain of six amino acids (Scanlan et al., 1994; Goldstein et al., 1997; Pineiro-Sanchez et al., 1997). FAP-α has been known to be shed from the cell-surface and recent work has identified a serum form of FAP-α (Collins et al., 2004; Lee et al., 2004). In its membrane-bound form, most of the protein, including the catalytic domain, is exposed to the extracellular environment (Fig. 1 from Cheng et al., 2002). The catalytic domain consists of the catalytic serine (S624) flanked by glycines in a classical consensus sequence for an active site serine, GWSYG. The catalytic serine in conjunction with aspartate (D702) and histidine (H734) comprise the catalytic triad (Scanlan et al., 1994; Goldstein et al., 1997; Pineiro-Sanchez et al., 1997). The structure of murine FAP-α was modeled based on the crystal structure of prolyl oligopeptidase, a related post-prolyl peptidase (Cheng et al., 2002) as was done earlier for human DPPIV (Gorrell et al., 2001) (Fig. 1). The model postulates that the extracellular portion of FAP-α consists of a seven-bladed β propeller domain sitting on top of an αβ hydrolase domain that includes the catalytic triad (Cheng et al., 2002). A flexible pore in the β propeller domain may serve as a filter to selectively admit proteins to the catalytic domain (Gorrell et al., 2001; Cheng et al., 2002) (Fig. 1).

The β propeller (amino acids 99-499 of FAP-α) may also determine substrate and extracellular matrix binding. Notably, the extracellular matrix adhesion of rat DPPIV occurs through binding of amino acids 313-319 (LQWLKRRI) to fibronectin (Cheng et al., 1998) and through binding of amino acids 236–491 to type I collagen (Lèoster et al., 1995). Although FAP-α has a domain (306-314, LQWLKRVQ) that differs from the fibronectin binding domain of rat DPPIV in only the last two amino acids, as yet there is no evidence that FAP-α binds fibronectin.

The β propeller of FAP-α could be important for forming complexes between FAP-α and other membrane-bound molecules. For example, α integrins are another class of cell-surface molecules that possess a seven-bladed β propeller. The α3β1 integrin has a β-propeller region in the α3 chain that includes the binding site for laminin-5, a known adhe-