Regulation of dipeptidyl peptidase 8 and 9 expression in activated lymphocytes and injured liver

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Abstract

AIM: To investigate the expression of dipeptidyl peptidase (DPP) 8 and DPP9 in lymphocytes and various models of liver fibrosis.

METHODS: DPP8 and DPP9 expression were measured in mouse splenic CD4+ T-cells, CD8+ T-cells and B-cells (B220+), human lymphoma cell lines and mouse splenocytes stimulated with pokeweed mitogen (PWM) or lipopolysaccharide (LPS), and in dithiothreitol (DTT) and mitomycin-C treated Raji cells. DPP8 and DPP9 expression were measured in epidermal growth factor (EGF) treated Huh7 hepatoma cells, in fibrotic liver samples from mice treated with carbon tetrachloride (CCl4) and from multidrug resistance gene 2 (Mdr2/Abcb4) gene knockout (gko) mice with biliary fibrosis, and in human end stage primary biliary cirrhosis (PBC).

RESULTS: All three lymphocyte subsets expressed DPP8 and DPP9 mRNA. DPP8 and DPP9 expression were upregulated in both PWM and LPS stimulated mouse splenocytes and in both Jurkat T- and Raji B-cell lines. DPP8 and DPP9 were downregulated in DTT treated and upregulated in mitomycin-C treated Raji cells. DPP9-transfected Raji cells exhibited more annexin V+ cells and associated apoptosis. DPP8 and DPP9 mRNA were upregulated in CCl4 induced fibrotic livers but not in the lymphocytes isolated from such livers, while DPP9 was upregulated in EGF stimulated Huh7 cells. In contrast, intrahepatic DPP8 and DPP9 mRNA expression levels were low in the Mdr2 gko mouse and in human PBC compared to non-diseased livers.

CONCLUSION: These expression patterns point to biological roles for DPP8 and DPP9 in lymphocyte activation and apoptosis and in hepatocytes during liver disease pathogenesis.

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Key words: Dipeptidyl peptidase; CD26; Lymphocytes; Liver fibrosis; Biliary fibrosis

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INTRODUCTION

The four enzyme members of the dipeptidyl peptidase (DPP) 4 gene family, DPP4, fibroblast activation protein (FAP), DPP8 and DPP9, have attracted considerable research interest in recent years since DPP4 inhibitors became a successful therapy for type 2 diabetes\(^{[6,7]}\). DPP4, the most well characterized family member, has ubiquitous cell surface and extracellular expression\(^{[2,4-7]}\). DPP8 and DPP9 are the most recently discovered members of the DPP4 gene family\(^{[8-11]}\). DPP4, DPP8 and DPP9 are ubiquitously expressed cytosolic enzymes with DPP4-like activity\(^{[8,11,12]}\). They are expressed by major epithelial organs including liver, colon, small intestine, stomach, lung, skin, tongue, kidney, testis and the lymphoid cells of lymph node, blood, thymus, and spleen\(^{[13]}\). The biological functions of DPP8 and DPP9 are largely uncharacterized.

DPP4 is also known as CD26 and has important roles in the immune system. It is a costimulatory molecule in T cell activation and proliferation and is critical in the development of T helper 1 responses to foreign antigens. It is expressed at detectable levels by some resting T cells but the cell surface expression increases 5-10 fold following stimulation with antigen or anti-CD3 antibodies\(^{[1,2,23,24]}\). In support of this viewpoint, there is some evidence that DPP8 and DPP9 are functionally significant in the immune system. Their mRNA levels are elevated in activated human leukocytes\(^{[25,26]}\). An inhibitor of DPP8 and DPP9 attenuates proliferation in \textit{in vitro} models of human T-cell activation\(^{[25]}\). An inhibitor selective for DPP8 and DPP9 versus related proteases can suppress DNA synthesis in mitogen-stimulated splenocytes from both wildtype DPP4+/- and DPP4/- gene knockout (gko) mice\(^{[27]}\). Moreover, DPP8 and DPP9 have been implicated in hematopoiesis and in inflammatory diseases including arthritis\(^{[2,3,28,29]}\). Most importantly, DPP8 and DPP9 are involved in processing and degradation of peptides involved in antigen presentation by Major histocompatibility complex class I\(^{[30]}\).

Inflammatory and immune responses are important in liver injury. Improved understanding of immune response, inflammation and fibrogenic progression is needed to advance the understanding of liver disorders. DPP8 and DPP9 are expressed in hepatocytes and lymphocytes of human cirrhotic liver\(^{[13]}\). Hepatocytes in the perisepal area of regenerative nodules and lymphocytes in the portal tracts are strongly positive for DPP8 and DPP9 \textit{in situ} hybridization (ISH)\(^{[13]}\). Bile ducts and ductular reactions are ISH positive for DPP9 but not for DPP8\(^{[3,4]}\). However, the role of DPP8 and DPP9 in liver is unknown. Other members of this protease family, DPP4 and FAP, are altered in liver diseases and are potential disease markers and therapeutic targets\(^{[31-36]}\). Despite the pleiotropic roles of DPP4 and FAP in various biological processes, DPP4 and FAP gko mice exhibit no spontaneous defects, suggesting that DPP4 and FAP are not essential for normal functions, and hence, targeting them is likely to lack adverse side effects\(^{[37,38]}\).

DPP8 and DPP9 have interesting properties in cell biological processes that may contribute to disease pathogenesis, such as apoptosis and cell migration\(^{[39,40]}\). Their biological functions, especially in the immune system, are important considerations for the selectivity of DPP4 inhibitors over DPP8 and DPP9 in clinical development of DPP antagonists. Here we studied the expression of DPP8 and DPP9 in lymphocyte activation, proliferation and apoptosis and in liver injury to elucidate their potential biological roles in the immune system and in liver diseases.

MATERIALS AND METHODS

Materials

Antibodies are detailed in Table 1. Other materials were from Sigma-Aldrich (St Louis, MO, United States) unless stated.

Animal studies

Mice were maintained in the Centenary Institute animal facility under specific pathogen-free conditions. The Animal Ethics Committee of the University of Sydney approved experimental procedures and housing arrangements. FAP gko\(^{[38]}\) and DPP4 gko mice\(^{[41]}\) (C57BL/6j background) were bred at the Animal Resource Centre (Perth, Australia). Female multidrug resistance gene 2 (\textit{Mdr2} / Abhd4) gko mice (FVB/N background) with targeted disruption of \textit{Mdr2}, were obtained from Jackson Laboratory (Jackson Laboratory, Bar Harbor, ME, United States)\(^{[35]}\). Live samples from the \textit{Mdr2} gko and wild type (wt) mice were obtained at 4, 8 and 12 wk after birth, the time points that span the most active fibrosis progression\(^{[37]}\). RNA was obtained as previously described\(^{[41]}\). Lymphocytes from wt, DPP4 gko and FAP gko mice spleen, liver and lymph nodes were isolated as previously described\(^{[41]}\).

For the liver fibrosis mouse model, 8-wk-old female wt, DPP4 gko and FAP gko mice were injected intra-peritoneally with carbon tetrachloride (CCL\(_4\)) twice weekly for 3 wk. Each dose comprised 5.36 µL of 12% CCL\(_4\) (in paraflin oil) per gram of initial weight of each mouse. Significantly elevated alanine aminotransferase (ALT) (68 ± 11.1 U/L vs untreated controls 32 ± 1.2 U/L) indicated liver injury. ALT was performed by an auto-analyzer at the Clinical Biochemistry Department of the Royal Prince Alfred Hospital. Organs were collected 3 d after the final CCL\(_4\) treatment.
Table 1  Antibodies used in immunoblot and flow cytometry

| Name                        | Isotype          | Conjugate | Dilution | Supplier                        | Catalogue no. |
|-----------------------------|------------------|-----------|----------|---------------------------------|---------------|
| Primary antibodies          |                  |           |          |                                 |               |
| CD4                         | Rat IgG          | FITC      | 1:50     | BD Pharmingen, NJ, United States | 557307        |
| B20                         | Rat IgG          | PE        | 1:50     | CalTag Laboratories, CA, United States | RM2604-3     |
| CD8                         | Rat IgG          | APC       | 1:50     | BD Pharmingen, NJ, United States | 553035        |
| Annexin V                   |                  | APC       | 1:50     | BD Pharmingen, NJ, United States | 550474        |
| V5                          | Mouse monoclonal IgG |        | 1:5000  | Invitrogen                      | R960-25       |
| DPP9-catalytic domain       | Rabbit polyclonal|          | 1:2000   | Abcam Inc.                      | Ab42077       |
| DPP8-catalytic domain       | Rabbit polyclonal|          | 1:2000   | Abcam Inc.                      | Ab42076       |
| GAPDH                       | Mouse monoclonal |          | 1:1000   | EnCor biotechnology             | MCA-1D4       |
| β-actin                     | Rabbit polyclonal|          | 1:1000   | Sigma                           | A2103         |
| Anti-rabbit IgG             | Goat             | HRP       | 1:3000   | DAKO, Carpathia, CA, United States | PO448         |
| Anti-mouse                  | Goat             | R-PE      | 1:400    | Molecular Probes                | P582          |

Human liver samples

Human liver tissues were obtained from liver transplant recipients in accordance with National Health and Medical Research Council guidelines under Royal Prince Alfred Hospital Human Ethics Committee approvals. Non-diseased liver donors had an age range of 56-58 years and mixed genders. Cirrhotic livers were from primary biliary cirrhosis (PBC) patients of average age 51.7 ± 13.3 years (range 27-67 years; 10 females, 2 males) and end stage alcoholic liver disease patients of average age 49.3 ± 8 years (range 34-60 years, 9 males) as described previously[40].

In vitro stimulation assays

Human B lymphocyte Burkitt's lymphoma cell line (Raji) (ATCC, CCL-86) and human T cell leukemia cell line (Jurkat) (ATCC, TIB-153) were cultured in Roswell Park Memorial Institute (RPMI) Medium 1640 (Invitrogen, Carlsbad, CA, United States) supplemented with 10% fetal calf serum (FCS) and Penicillin-Streptomycin (100 units of penicillin and 100 μg/mL of streptomycin) (1 × P/S) and human liver hepatocellular carcinoma cell line HuH7 were grown in Dulbecco's Modified Eagle's Medium (Invitrogen) supplemented with 10% FCS and 1 × P/S.

Lymphocytes at 1 × 10⁶ cells/mL RPMI were treated with either 5 μg/mL pokeweed mitogen (PWM), 20 μg/mL lipopolysaccharide (LPS), 50 μg/mL Mitomycin C or 10 mmol/L dithiothreitol (DTT). Human liver hepatocellular carcinoma cell line, HuH7 cells were serum starved for 20 h before stimulation with 0, 1, 10, 100 ng/mL epi- dermal growth factor (EGF; R-D Systems, MN, United States) for 4 h.

Apoptosis assay

To determine if DPP9 overexpression induces apoptosis, Raji cells were transiently transfected with wtDPP9-V5-His, mutDPP9-V5-His or vector control (pcDNA3.1/2b/V5-HisA; Invitrogen, Carlsbad, CA, United States) as described previously[40], then cultured. The lymphocytes were transfected by electroporation using Amaxa® Cell Line Nucleofector® Kit V (Lonza, Basel, Switzerland) on a Lonza-amaxa Nucleofector device (Lonza). Forty hours post transfection, cells were washed with annexin binding buffer (10 mmol/L HEPES, 140 mmol/L NaCl, 2.5 mmol/L CaCl₂, pH 7.4). Staining involved incubating cells with annexin V antibody (Table 1) for 30 min at room temperature in the dark followed by 4',6-diamidino-2-phenylindolindole (DAP), Sigma Aldrich) at 100 ng/mL. Cells were enumerated using flow cytometry. Analysis was performed using FlowJo software (Tree Star Inc., Ashland, OH, United States).

Fluorescence activated cell sorting

To isolate mouse lymphocyte subsets, 3 × 10⁶ splenocytes were resuspended in primary antibody diluted in phosphate buffered saline (PBS) containing 1% FCS and incubated in the dark, on ice for 30 min. The primary antibodies used are listed in Table 1. Following antibody staining, cells were washed with PBS containing 1% FCS. Cells underwent a final resuspension of 2 × 10⁷ cells/mL of PBS with 5% FCS and 2 mmol/L ethylene diamine tetraacetic acid (EDTA) to minimize clumping of cells. Twenty-five μL/mL of DAPI was added prior to cell sorting. Cell sorting was performed using the Fluorescence Activated Cell Sorting Vantage™ SE (BD Bioscience, NJ, United States). Cells were gated to exclude doublets and DAPI+ (dead) cells. Three-way sort was performed to collect CD4+ cells, CD8+ cells and B220+ cells into separate collection tubes.

Real time quantitative polymerase chain reaction

RNA from cells was extracted using the RNAquick® Micro™ kit (Ambion, TX, United States) following manufacturer’s instructions. Total RNA (1 μg) was then reverse-transcribed to cDNA using 10 pmol of oligo(dT)₁₂₋₁₈ primer (Invitrogen, Carlsbad, CA, United States), 10 mmol/L deoxyribonucleotide triphosphates and SuperScript III reverse transcriptase (Invitrogen). Real time quantitative poly-

DPP: Dipeptidyl peptidase; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; IgG: Immunoglobulin G; FITC: Fluorescein; PE: Phycoerythrin; APC: Allophycocyanin; HRP: Horse radish peroxidase; R-PE: R-phycoerythrin.
mRNA levels corresponded to protein expression, DPP8 and DPP9 proteins were measured in Jurkat cells stimulated in vitro with PWM. Both DPP8 and DPP9 were upregulated in a time dependent manner (Figure 2B and C).

Similarly, mRNA levels of DPP8 and DPP9 were upregulated in LPS stimulated mouse splenocytes (Figure 3A). Also, LPS stimulated Raji (B cells) had upregulated DPP8 and DPP9 protein expression in a time dependent manner (Figure 3B and C).

Immunoblotting assay
Cells were washed with ice-cold PBS three times and then lysed with ice-cold lysis buffer (50 mmol/L Tris-HCl, 1 mmol/L EDTA, 1 mmol/L MgCl₂, 300 µL of 150 mmol/L NaCl, 1% Triton-114, 10% glycerol and 1 × Roche complete protease inhibitor cocktail (Roche Applied Science, Mannheim, Germany) and stored at -80 °C. Protein concentration was determined using the micro BCA protein assay kit (Thermo Scientific, CA, United States) following the manufacturer’s protocol. 50 µg total of each cell lysate in LDS sample buffer (catalogue No. NP0007, Invitrogen) with reducing agent (catalogue No. NP0004, Invitrogen) in conditions that retain DPP8 and DPP9 dimerization was resolved on 3%-8% Tris-acetate sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Invitrogen) followed by immunoblotting.

**RESULTS**

To investigate which lymphocyte subsets express DPP8 and DPP9, their transcript levels were quantified in the major lymphocyte subpopulations, CD4⁺ (helper) and CD8⁺ (cytotoxic) T cells and B220⁺ (B cells) from normal C57BL/6 mouse splenocytes. All three lymphocyte subsets expressed DPP8 and DPP9 mRNA (Figure 1). DPP8 and DPP9 transcripts were expressed to significantly greater levels than DPP4 transcripts in CD4⁺ T cell subpopulation (P = 0.02) and DPP9 mRNA was significantly more abundant than DPP4 mRNA in B cells (P = 0.03).

To examine whether, like DPP4, DPP8 and DPP9 are upregulated upon lymphocyte activation, mouse splenocytes were stimulated in vitro with PWM[8,43] and LPS[44,47]. DPP8 and DPP9 mRNA was markedly upregulated in PWM stimulated mouse splenocytes in a time dependent manner (Figure 2A). To examine whether the increased mRNA levels corresponded to protein expression, DPP8 and DPP9 proteins were measured in Jurkat (T cells) stimulated in vitro with PWM. Both DPP8 and DPP9 were upregulated in a time dependent manner (Figure 2B and C).

Immunoblots of DPP8 exhibited a slow mobility band at 150-180 kDa, which probably represents dimer or processed dimer, in addition to the faster mobility bands at 95-100 kDa that are likely to be monomer and truncated or trimmed monomer (Figure 2B and 3B). DPP9 showed a slow mobility band of monomer at 110 kDa and faster mobility bands, which are possibly truncated or trimmed monomers at 75-95 kDa (Figure 2C and 3C)[8,40,47]. The intensity of all three DPP8 bands increased in a time dependent manner with PWM stimulation in Jurkat cells (Figure 2B). However, in LPS stimulated Raji cells the intensity of only the 150 kDa band increased in a time dependent manner (Figure 3B). The intensity of all the DPP9 bands increased with time in both PWM stimulated Jurkat cells and LPS stimulated Raji cells (Figure 2C and 3C). In PWM stimulated Jurkat cells, DPP8 and DPP9 expression both peaked at 48 h (Figure 2B and C). In Raji cells, increased expression of DPP8 was observed at 72 h post LPS stimulation (the longest time point of the
Figure 2  Dipeptidyl peptidase 8 and dipeptidyl peptidase 9 upregulation in pokeweed mitogen stimulated lymphocytes. A: Dipeptidyl peptidase (DPP) 8 and DPP9 mRNA in mouse splenocytes (representative data from one of three mice); DPP8 and DPP9 proteins from Jurkat cells; B: Immunoblot of DPP8 and densitometry analysis of DPP8 bands; C: Immunoblot of DPP9 and densitometry analysis of bands. Densitometry data shown are relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). PWM: Pokeweed mitogen.

Figure 3  Dipeptidyl peptidase 8 and dipeptidyl peptidase 9 upregulation in lipopolysaccharide stimulated lymphocytes. A: Dipeptidyl peptidase (DPP) 8 and DPP9 mRNA in mouse splenocytes (representative data from one of three mice); B: Immunoblot of DPP8 and densitometry analysis of DPP8 bands; C: DPP9 immunoblot and densitometry analysis of DPP9 bands relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). LPS: Lipopolysaccharide.
study) (Figure 3B), and $DPP9$ expression peaked at 60 h (Figure 3C).

**DPP8 and DPP9 in lymphocyte apoptosis**

We have previously shown that $DPP9$ overexpression induces intrinsic cell apoptosis in human hepatoma and embryonic kidney cell lines$^{[39,40]}$. Similar to epithelial cells, $DPP9$ overexpression induced increased cell death in Raji cells (Figure 4A). This effect was less pronounced when Raji cells were transfected with mutant $DPP9$ lacking DSP activity (Figure 4A), suggesting that the enzyme activity of $DPP9$ influences lymphocyte apoptosis.

Interestingly, Raji cells treated with DTT, an antioxidant that impairs cell apoptosis, had less $DPP8$ and $DPP9$ expression compared to untreated cells (Figure 4B and C). Conversely, treatment of Raji cells with mitomycin C, a lectin that impairs cell proliferation, resulted in increased $DPP8$ and $DPP9$ expression in Raji cells (Figure 4B and C). Intensities of all $DPP8$ and $DPP9$ band sizes were less with DTT treatment and greater with Mitomycin C treatment compared to untreated cells (Figure 4B and C).

**DPP9 in EGF stimulated hepatocytes**

EGF is a regulatory factor in cell survival, growth, proliferation, and differentiation$^{[49]}$. Previously, we have shown that $DPP9$ overexpression impairs EGF-stimulated cell proliferation in HepG2 and Huh7 human hepatoma cell lines$^{[40]}$. $DPP9$ expression at 75 kDa was greater in Huh7 cells after EGF stimulation (Figure 5). This study expands the association of $DPP9$ with EGF in this hepatoma cell line.

**Intrahepatic DPP8 and DPP9 upregulation in CCl4 induced liver injury**

To examine $DPP8$ and $DPP9$ expression in liver injury,
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**DISCUSSION**

This study significantly promotes our understanding of the novel proteases DPP8 and DPP9 in lymphocytes, hepatocytes and liver injury. We showed that DPP8 and DPP9 are widely expressed in lymphocyte subpopulations and upregulated in mitogen activated lymphocytes in a time dependent manner. Besides lymphocyte activation, we demonstrated their potential involvement in lymphocyte apoptosis. In liver, we showed that DPP8 and DPP9 expression levels were altered in liver injury and confirmed their role in the regulation of EGF in hepatocytes, a mitogen that is considered crucial for hepatocyte proliferation and liver regeneration. The interestingly variable expression patterns of DPP8 and DPP9 in different conditions in lymphocytes and in liver injury suggest that these proteases may have important regulatory roles in the immune system and in liver disease pathogenesis.

CCL4 was used to induce liver fibrosis in wt, DPP4 gko and FAP gko mice. DPP4, DPP8 and DPP9 mRNA were significantly more abundant in the livers from CCL4 treated mice of all three genotypes compared to the untreated controls (Figure 6A). DPP8 and DPP9 mRNA expression in the CCL4 treated livers were greater in the FAP gko mice compared to wt (DPP8 P = 0.02; DPP9 P = 0.02), suggesting that DPP8 and DPP9 might have compensatory roles in the absence of FAP (Figure 6A). The increase in DPP9 mRNA in the fibrotic livers was consistent with protein expression in wt mice (P = 0.05) (Figure 6B). An appropriate antibody to mouse DPP8 is not available.

Since DPP8 and DPP9 are expressed by human hepatic lymphocytes[13] and because there is an increase of infiltrating lymphocytes in liver fibrosis, we examined whether the mouse hepatic lymphocytes were likely to contribute to the observed upregulation of DPP expression. However, DPP mRNA in the mouse hepatic lymphocytes was similar in the fibrotic and normal livers (Figure 6C).

**Intrahepatic DPP8 and DPP9 downregulation in biliary liver disease**

The Mdr2 gko mouse strain is deficient in the canalicular phospholipid flippase and is a model of periporal biliary fibrosis resembling primary sclerosing cholangitis[41]. These mice develop spontaneous hepatomegaly as early as 2 wk after birth and significant biliary fibrosis with a fivefold increased liver collagen content by 12 wk of age, when no further fibrosis progression occurs[42]. Measuring DPPs in these mice at 4, 8 and 12 wk of age showed that DPP mRNA expression was surprisingly very low at wk 4, significantly lower than in wt (DPP8 P = 0.03; DPP9 P = 0.03; DPP4 P = 0.03) (Figure 7A). At 8 and 12 wk of age, DPP expression levels were similar to wt.

In human end-stage PBC livers, DPP9 mRNA expression was significantly less than in the non-diseased livers (P = 0.03) (Figure 7B). This finding is consistent with the results in the Mdr2 gko mice and with the human DPP9 Western blot data[43]. DPP8 mRNA expression levels in the non-diseased and PBC livers were not statistically different (P = 0.057).

**Figure 6** Dipeptidyl peptidase mRNA upregulation in carbon tetrachloride induced liver injury. A: Multiple of intrahepatic mRNA in carbon tetrachloride (CCL4) treated mice to mean of untreated control mice; *P < 0.05 in CCL4 treated fibroblast activation protein (FAP) gene knockout (gko) vs wild type (wt); B: Dipeptidyl peptidase (DPP) 9 immunoblot of livers from CCL4 treated (lanes 1-6) and untreated mice (lanes 7-12) (n = 6 per group); Densitometry of intrahepatic DPP9 relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). *P < 0.05 vs untreated controls; C: mRNA quantitation from isolated hepatic lymphocytes relative to 18S.
**DPP8/9** activity and expression in lymphocytes have been reported previously, but which lymphocyte subpopulations express **DPP8** and **DPP9** remained unknown. Here we show that all the lymphocyte subpopulations tested, CD4⁺ T cells, CD8⁺ T cells and B220⁺ B cells express **DPP8** and **DPP9**. The wide expression of **DPP8** and **DPP9** in lymphocyte subpopulations suggests that these proteases have essential roles in the immune system. As it is now known that immune roles of **DPP4** are mainly extraenzymatic (such as protein-protein interaction), greater abundance of **DPP8** and **DPP9** compared to **DPP4/CD26** in the lymphocytes further supports the hypothesis that the immune effects with non-selective **DPP4** inhibitors in earlier studies were more likely due to **DPP8** and **DPP9** inhibition.

We demonstrated a quantitative time-dependent upregulation of **DPP8** and **DPP9** in mitogen-stimulated mouse splenocytes and human Jurkat CD4⁺ T cells, as well as in polyclonally activated Raji B cells. Therefore, **DPP8** and **DPP9** might have roles in both T and B cell activation. **DPP8** and **DPP9** were upregulated in lymphocytes following acute mitogen stimulation, but with prolonged stimulation, they were downregulated. Hence, the role of **DPP8** and **DPP9** perhaps differ in recently activated lymphocytes compared to persistently activated lymphocytes.

**DPP9** enzyme activity induces intrinsic cell apoptosis in epithelial cells through the phosphatidyl inositide-3-kinase/protein kinase B (Akt) signaling pathway. Our data on Raji cells suggest that **DPP9** could similarly have a role in intrinsic lymphocyte apoptosis. Moreover, the increase in **DPP8** and **DPP9** expression in mitomycin C treated cells is perhaps a hallmark of increased apoptosis in the absence of cell proliferation. **DPP9** substrates and ligands involved in these processes have not been identified.

The modulation of **DPP8** and **DPP9** expression with varying lymphocyte activation, proliferation and apoptosis, implies that **DPP8** and **DPP9** have important regulatory roles in lymphocytes that deserve further investigation. Their role in lymphocyte activation is likely to differ from that of **DPP4**. While the role of cell surface **DPP4** in lymphocyte proliferation appears to be mainly extra-enzymatic, enzyme inhibition of intracellular **DPP8** and **DPP9** affects lymphocyte proliferation. The observation of less annexin V staining in Raji cells overexpressing **DPP9** enzyme mutant compared to wild type **DPP9** suggests that enzyme activity of **DPP9** is important for its role in apoptosis. **DPP9** modulates Akt phosphorylation in hepatoma cell lines, so **DPP8** and **DPP9** might similarly modulate the activity of signaling molecules that are crucial in lymphocyte activation pathways. **DPP8** can cleave several chemokines, stromal cell-derived factor (SDF)-1α, SDF-1β, inflammatory protein 10 and interferon-inducible T-cell alpha chemoattractant, in vitro, however since **DPP8** is an intracellular protease, the biological relevance of this cleavage is unknown.

The association of **DPP4** and E-AP with liver fibrosis is well documented. Here we have demonstrated possible involvement of **DPP8** and **DPP9** in liver fibrosis, too. Treatment of mice with CCLs for 3 wk, which represents early fibrosis with mild hepatic injury, increased intrahepatic **DPP8** and **DPP9** expression. This association with early stage disease may suggest pro-fibrogenic roles of **DPP8** and **DPP9**. Though **DPPs** have been implicated in inflammation and inflammatory diseases, no change in **DPP** expression was observed in hepatic lymphocytes in this early stage fibrosis, suggesting that hepatocytes, which constitute more than 80% of the liver cell population, are probably the major source of upregulated **DPP8** and **DPP9** in this liver fibrosis model.

Unlike the CCLs induced liver fibrosis model, **DPP8** and **DPP9** were downregulated in end stage human PBC and in the Mdr2 gko mice. This suggests that **DPP8** and **DPP9** expression varies with the pathophysiology of liver diseases. The mouse CCLs model represents zone 3 fibrosis whereas Mdr2 gko represents a zone 1 fibrosis model. **DPP8** and **DPP9** show a zonal distribution pattern, with stronger staining in zone 3, the periportal hepatocytes and perportal lymphocytes. Hence, the zonal injury pattern may be important for **DPP8** and **DPP9** expression. Another possibility could be that activated cholangiocytes downregulate **DPP8** and **DPP9** expression. In the Mdr2 gko mice, **DPP8** and **DPP9** expression was least at week 4, when the cholangiocytes are...
most active. Hence, this could be the reason why DPP8 and DPP9 expression was downregulated in human PBC and Mdr2 gko mice.

Alternatively, the differential expression of DPP in the different liver diseases could be due to acute or chronic stimuli. CCl4 induces acute liver injury with hepatocyte damage followed by a repair phase that involves increased collagen deposition. Administration of CCl4 twice per week for 3 wk leads to repeated cycles of injury and repair that results in fibrosis. We collected liver samples from the CCl4 treated mice at day 3 after the last CCl4 injection. At day 3, hepatocyte apoptosis is waning whereas fibrosis is developing. In contrast, the Mdr2 gko mice and human end stage PBC represent chronic liver injury, whereby there is persistent (mild) hepatocyte damage, a fibrogenic cholangiocyte/progenitor cell response and downregulation of collagenolytic activity resulting in continuing progression of biliary fibrosis until week 12 of age. Thus, our data are consistent with the paradigm that DPP8 and DPP9 are upregulated in acute disease states then downregulated with progression to chronic disease states.

This distinctive DPP expression pattern in different liver diseases suggests that DPP8 and DPP9 have important regulatory roles in the pathogenesis of liver diseases, perhaps in modulating liver regeneration and apoptosis, which are important processes in liver disease progression. DPP9 impairs EGF-stimulated hepatoma proliferation. Our observation that DPP9 is upregulated in the presence of EGF is perhaps part of a regulatory mechanism of DPP9 in hepatocyte proliferation. DPP9 can induce intrinsic apoptosis in hepatoma cell lines via the Akt signaling pathway. Furthermore, DPP8 and DPP9 influence cell-extracellular matrix (ECM) interactions in vitro and in liver fibrogenesis, cell-ECM interaction is responsible for disrupting wound healing and progressive scarring in liver disease.

The upregulated expression of DPP8 and DPP9 in acute conditions and less expression in chronic or persistent conditions in the immune system and in liver injury suggests that DPP8 and DPP9 are crucial for early cellular responses to stimuli. The mechanisms of DPP8 and DPP9 are yet to be elucidated. One obstacle in DPP8 and DPP9 studies is the poor availability of appropriate tools, such as monoclonal antibodies and selective inhibitors.

In conclusion, our study suggests that DPP8 and DPP9 have fundamental roles in the immune system, in lymphocyte activation and in apoptosis and they could be involved in liver fibrogenesis. A better understanding of the biological functions of DPP8 and DPP9 could help reveal their therapeutic potential for liver diseases, cancer, inflammatory and autoimmune diseases.

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COMMENTS

Background

The four enzyme members of the dipeptidyl peptidase (DPP) 4 gene family, DPP4, fibroblast activation protein (FAP), DPP8 and DPP9 have attracted considerable research interest in recent years since DPP4 inhibitors became a successful therapy for type 2 diabetes and FAP a potential cancer therapeutic target. DPP8 and DPP9 are the more recently discovered members of the DPP4 gene family. They are ubiquitously expressed cytoplasmic enzymes with DPP4-like enzyme activity. Many compounds intended to inhibit DPP4 or FAP also inhibit DPP8 and DPP9, but the compounds that became successful diabetes drugs are DPP4-selective.

Research frontiers

DPP4 is also known as CD26 T cell differentiation marker and has roles in T cell activation and proliferation. DPP8 and DPP9 are in lymphoid tissues and may have functional significance in the immune system. DPP8 and DPP9 are expressed in hepatocytes and expression is elevated in damaged hepatocytes near the septum of human cirrhotic liver. However, potential roles of DPP8 and DPP9 in liver disease are unknown. Here the authors studied the expression of DPP8 and DPP9 in lymphocyte activation, proliferation and apoptosis and in liver injury models to elucidate their potential biological roles in the immune system and in liver diseases. Models included hepatotoxicity from CCl4, and the multidrug resistance gene 2 knockout mice that spontaneously develop biliary fibrosis.

Innovations and breakthroughs

This study significantly promotes our understanding of the novel proteases DPP8 and DPP9 in lymphocytes, hepatocytes and liver injury. The authors showed that DPP8 and DPP9 were widely expressed in lymphocyte subpopulations and were upregulated in activated lymphocytes in a time dependent manner. The authors also demonstrated potential involvement of DPP8 and DPP9 in lymphocyte apoptosis. In liver, the authors showed that DPP8 and DPP9 expression levels were altered in liver injury and confirmed their role in the regulation of epidermal growth factor in hepatocytes, a mitogen that is considered crucial for hepatocyte proliferation and liver regeneration.

Applications

This study suggests that DPP8 and DPP9 have fundamental roles in the immune system, in lymphocyte activation and in apoptosis and they could be involved in chronic liver injury pathogenesis.

Terminology

DPP4 enzyme activity is a specialized proteolytic enzyme activity that cuts two amino acids from the N-terminus of each target peptide, usually cutting after a proline residue; Lymphocyte activation is a cellular process that leads to a radical shift in cell behavior to a more active and proliferative one. The activation of lymphocytes serves two purposes: augmenting the number of cells to respond to a particular antigen (clonal expansion), and specializing to produce cytokines, and produce antibodies against a pathogen; cell apoptosis is the process of cell death mediated by an intracellular program. Apoptosis is important for normal cell turnover and organ remodeling.

Peer review

The manuscript deals with regulation of DPP8 and DPP9 expression in activated lymphocytes and injured liver. Here the authors focus on the expression levels of DPP8 and DPP9 in lymphocyte subpopulations in a time dependent manner. The authors have confirmed the altered expression level of DPP8 and DPP9 in liver injury and also confirmed their role in the regulation of epidermal growth factor in hepatocytes. The work has been carefully conducted and the experiments are clearly described in the vast majority of the cases.

REFERENCES

1 Rosenblum JS, Kozarich JW. Prolyl peptidases: a serine protease subfamily with high potential for drug discovery. Curr Opin Chem Biol 2003; 7: 496-504 [PMID: 12941425 DOI: 10.1016/S1367-5931(03)00084-X]

2 Yu DM, Yao TW, Chowdhury S, Nadvi NA, Osborne B, Chur-
ch WB, McCaughan GW, Gorrell MD. The dipeptidyl peptidase IV family in cancer and cell biology. FBS J 2010; 277: 1126-1144 [PMID: 20704209 DOI: 10.1569/jhc.2009.935760]

Keane FM, Chowdhury S, Yao T-W, Nadvi NA, Gall MG, Chen Y, Pham D, Vieira de Ribeiro AJ, Church WB, McCaughan GW, Gorrell MD, Yu DMT. Targeting dipeptidyl peptide-4 (DPP-4) and fibroblast activation protein (FAP) for diabetes and cancer therapy. In: Dunn B, editor. Proteins as drug targets. Cambridge, UK: Royal Society of Chemistry, 2011: 119-145

Gossrau R. [Peptidases II. Localization of dipeptidylpeptidase IV (DPP IV). Histochemical and biochemical study]. Histochemistry 1979; 60: 251-248 [PMID: 457448 DOI: 10.1007/BF00495756]

Hartel S, Gossrau R, Hanksi C, Reutter W. Dipeptidyl peptidase (DPP) IV in rat organs. Comparison of immunohistochemistry and activity histochemistry. Histochemistry 1988; 89: 151-161 [PMID: 2456278 DOI: 10.1007/BF00489918]

McCaughan GW, Wickson JE, Cresswick PF, Gorrell MD. Identification of the bile canaliculal cell surface molecule GP110 as the ectopeptidase dipeptidyl peptidase IV: an analysis by tissue distribution, purification and N-terminal amino acid sequence. Hepatology 1990; 11: 534-544 [PMID: 1970322 DOI: 10.1002/hep.1840110403]

Gorrell MD, Gysbers V, McCaughan GW. CD26: a multi-functional integral membrane and secreted protein of actinomycetes. FEBS Lett 2000; 582: 819-825 [PMID: 18275857 DOI: 10.1016/S0014-5793(00)01059-4]

QI SY, Riviere PJ, Trojan J, Junien JL, Akinsanya KO. Cloning and characterization of dipeptidyl peptidase 10, a new member of an emerging subgroup of serine proteases. Biochem J 2001; 357: 277-292 [PMID: 11662155]

Ajami K, Abbott CA, McCaughan GW, Gorrell MD. Dipeptidyl peptidase 9 has two forms, a broad tissue distribution, cytoplasmic localization and DP4-like peptidase activity. Biochim Biophys Acta 2004; 1679: 18-28 [PMID: 15245913 DOI: 10.1016/j.bjba.2004.03.010]

Olsen C, Wagtmann N. Identification and characterization of human DPP8, a novel homologue of dipeptidyl peptidase IV. Gene 2002; 299: 185-193 [PMID: 12459266 DOI: 10.1016/S0378-1119(02)01059-4]

Yu DM, Ajami K, Gall MG, Park J, Menz RL, Starr AE, Cox JH, Abbott CA, Overall CM, Gorrell MD. Stromal cell-derived factors 1alpha and 1beta, inflammatory protein-10 and interferon-inducible T cell chemotactic are novel substrates of dipeptidyl peptidase 8. FBS Lett 2008; 582: 819-825 [PMID: 18275857 DOI: 10.1016/j.flet.2008.02.005]

Yu DM, Ajami K, Gall MG, Park J, Lee CS, Evans KA, McLaughlin EA, Pitman MR, Abbott CA, McCaughan GW, Gorrell MD. The in vivo expression of dipeptidyl peptidases 8 and 9. J Histochem Cytochem 2009; 57: 1025-1040 [PMID: 19581630]

Fleischer B. A novel pathway of human T cell activation via a 103 KD T cell activation antigen. J Immunol 1987; 138: 1346-1350 [PMID: 3106357]

Gorrell MD, Miller HR, Brandon MR. Lymphocyte phenotypes in the abdominal mucosa of sheep infected with Haem- monchus contortus. Parasite Immunol 1988; 10: 661-674 [PMID: 321727]

Heike M, Möbius U, Knuth A, Meuer S, Meyer zum Büschenfelde KH. Tissue distribution of the T cell activation antigen Ta1. Serological, immunohistochemical and biochemical investigations. Clin Exp Immunol 1988; 74: 431-434 [PMID: 2466591]

Marguet D, Bernard AM, Vivier I, Darmoul D, Naquet P, Pierres M. cDNA cloning for mouse thymocyte-activating molecule. A multifunctional eco-dipeptidyl peptidase IV (CD26) included in a subgroup of serine proteases. J Biol Chem 1992; 267: 2280-2288 [PMID: 13708131]

Schnö P, Ansergue S. Dipeptidyl peptidase IV in the immune system. Cyttofluorimetric evidence for induction of the enzyme on activated T lymphocytes. Biochim Biophys Acta 1993; 1179: 699-707 [PMID: 1698386 DOI: 10.1015/bchm3.1993.701.2.699]

Kahne T, Lendeckel U, Wrenger S, Neubert K, Ansergue S, Reinhold D. Dipeptidyl peptidase IV: a cell surface peptidase involved in regulating T cell growth (review). Int J Mol Med 1999; 4: 3-15 [PMID: 10376311]

Hühn J, Ehrlich S, Fleischer B, von Bonin A. Molecular analysis of CD26-mediated signal transduction in T cells. Immunol Lett 2000; 72: 127-132 [PMID: 10841948 DOI: 10.1016/S0165-2478(00)01070-X]

Kirby M, Yu DM, O’Connor S, Gorrell MD. Inhibitor selectivity in the clinical application of dipeptidyl peptidase-4 inhibition. Clin Sci (Lond) 2010; 118: 31-41 [PMID: 19780719 DOI: 10.1042/CS20090047]

Yu DM, Slaitini L, Gysbers V, Riekhoff AG, Kahne T, Knott HM, De Meester J, Abbott CA, McCaughan GW, Gorrell MD. Soluble CD26/dipeptidyl peptidase IV enhances human lymphocyte proliferation in vitro independent of dipeptidyl peptidase enzyme activity and adenosine deaminase binding. Scand J Immunol 2011; 73: 102-111 [PMID: 21987570 DOI: 10.1111/j.1365-3083.2010.02488.x]

Lankas GR, Letting B, Roy RS, Eiermann GJ, Beconi MG, Biftu T, Chan CC, Edmondson S, Feeney WP, He H, Ippolito DE, Kim D, Lyons KA, Ok HO, Patel RA, Petrov AN, Pyyro KA, Qian X, Reigle L, Woods A, Wu JK, Zaller D, Zhang X, Zhu L, Weber AE, Thornberry NA. Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: potential importance of selectivity over dipeptidyl peptidases 8 and 9. Diabetes 2005; 54: 2988-2994 [PMID: 16186403 DOI: 10.2337/diabetes.54.10.2988]

Gorrell MD. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin Sci (Lond) 2005; 108: 277-292 [PMID: 15584901 DOI: 10.1042/CS20040032]

Abbott CA, Yu D, McCaughan GW, Gorrell MD. Post-proline-cleaving peptidases having DP IV like enzyme activity. Post-proline peptidases. Adv Exp Med Biol 2000; 477: 103-109 [PMID: 10849735]

Bank U, Heinburg A, Wohlfarth A, Koch G, Nordhoff K, Julius H, Helmut M, Breyer D, Reinhold T, Asserhe N. Suppression of arthritis by the inhibitors of dipeptidyl peptidase IV (DP IV)-like enzymes in T lymphocyte activation: investigations in DP IV/CD26-knockout mice. Clin Chem Lab Med 2009; 47: 268-274 [PMID: 19676138 DOI: 10.1515/CCLM.2009.062]

von Bonin A, Hühn J, Fleischer B. Dipeptidyl-peptidase-IV/ CD26 on T cells: analysis of an alternative T-cell activation pathway. Immunol Res 1998; 161: 43-53 [PMID: 9553763 DOI: 10.1111/j.1600-065X.1998.tb01570.x]

Reinhold D, Gohl A, Wrenger S, Reinhold A, Kühlmann UC, Faust J, Neubert K, Thielitz A, Brocke S, Täger M, Asserhe S, Bank U. Role of dipeptidyl peptidase IV (DP IV)-like enzymes in T lymphocyte activation: investigations in DP IV/CD26-knockout mice. Clin Chem Lab Med 2009; 47: 268-274 [PMID: 19676138 DOI: 10.1515/CCLM.2009.062]

Matsumoto Y, Bishop GA, McCaughan GW. Altered zonal
expression of the CD26 antigen (dipeptidyl peptidase IV) in human cirrhotic liver. *Hepatology* 1992; 15: 1048-1053 [PMID: 1350563 DOI: 10.1002/hep.1840150361]

32 Sniecka BA, Nardo B, Checo P, Mazzotti A, Bolondi L, Cavallari A. Aberrant dipeptidyl peptidase IV (DPP IV/CD26) expression in human hepatocellular carcinoma. *J Hepatol* 1997; 27: 337-345 [PMID: 9288609 DOI: 10.1016/S0168-8278 (97)00180-8]

33 Levy MT, McLaughan GW, Abbott CA, Park JE, Cunningham AM, Müller E, Rettig WJ, Gorrell MD. Fibroblast activation protein: a cell surface dipeptidyl peptidase and gelatinase expressed by stellate cells at the tissue remodelling interface in human cirrhosis. *Hepatology* 1999; 29: 1768-1778 [PMID: 10347120 DOI: 10.1002/hep.510290631]

34 Cox G, Kable E, Jones A, Fraser I, Manconi F, Gorrell MD. 3-dimensional imaging of collagen using second harmonic generation. *J Struct Biol* 2003; 141: 53-62 [PMID: 12576020 DOI: 10.1016/S1074-4747(02)00576-2]

35 Wang XM, Yu DM, McLaughan GW, Gorrell MD. Fibroblast activation protein increases apoptosis, cell adhesion, and migration by the LX-2 human stellate cell line. *Hepatology* 2005; 42: 935-945 [PMID: 16175601 DOI: 10.1002/hep.20853]

36 Lo L, McMennan SV, Williams PF, Bonner J, Chowdhury S, McLaughan GW, Gorrell MD, Yue DK, Twigg SM. Diabetes is a progression factor for hepatic fibrosis in a high fat fed mouse obesity model of non-alcoholic steatohepatitis. *J Hepatol* 2011; 55: 435-444 [PMID: 21184785 DOI: 10.1016/j.jhep.2010.10.039]

37 Marguet D, Baglio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribel U, Watanabe T, Drucker DJ, Wagtman N. Enhanced insulin secretion and improved glucose tolerance in mice lacking DCD26. *Proc Natl Acad Sci USA* 2000; 97: 6874-6879 [PMID: 10823914 DOI: 10.1073/pnas.120069197]

38 Niedermeyer J, Kriz M, Hilberg F, Garin-Chesa P, Bamberger U, Lenter MC, Park J, Viertel B, Püschnner H, Mauz M, Rettig WJ, Schnapp A. Targeted disruption of mouse fibroblast activation protein. *Mol Cell Biol* 2000; 20: 1089-1094 [PMID: 10920066 DOI: 10.1128/MCB.20.3.1089-1094.2000]

39 Yu DM, Wang XM, McLaughan GW, Gorrell MD. Extrahepatic functions of the dipeptidyl peptidase IV-related proteins DFP and DPP4 in cell adhesion, migration and apoptosis. *FEBS J* 2006; 273: 2447-2460 [PMID: 16704418 DOI: 10.1111/j.1742-4658.2006.05253.x]

40 Yao TW, Kim WS, Yu DM, Sharbeen G, McLaughan GW, Choi KY, Xia P, Gorrell MD. A novel role of dipeptidyl peptidase 9 in epidermal growth factor signaling. *Mol Cancer Res* 2011; 9: 948-959 [PMID: 21626264 DOI: 10.1158/1541-7786.MCR-10-0272]

41 Popov Y, Patsoner E, Hickert P, Trauner M, Schuppner D. Mdrr2 (Abcc4)-/- mice spontaneously develop severe biliary fibrosis via massive dysregulation of pro- and antifibrogenic genes. *J Hepatol* 2005; 43: 1045-1054 [PMID: 16223543 DOI: 10.1016/j.jhep.2005.06.025]

42 Holz LE, Benseler V, Bowon DG, Bouillet P, Strasser A, O'Reilly L, d'Avigdor GM, Bishop AG, McLaughan GW, Bertolino D. Intrahepatic murine CDS T-cell activation associates with a distinct phenotype leading to Bim-dependent death. *Gastroenterology* 2008; 135: 989-997 [PMID: 18619445 DOI: 10.1053/j.gastro.2008.05.078]

43 Wallays G, Ceuppens JL. Human T lymphocyte activation by pokeweed mitogen induces production of TNF-alpha and GM-CSF and helper signaling by IL-1 and IL-6 results in IL-2-dependent T cell growth. *Eur J Immunol* 1993; 4: 269-277 [PMID: 8266417]

44 Barcellini W, Sguotti C, Dall’Aglio P, Garelli S, Meroni PL. In vitro immunoglobulin synthesis: T-cell requirement in pokeweed and staphylococcus aureus B-cell activation. *J Clin Lab Immunol* 1985; 17: 177-181 [PMID: 3877812]

45 Serke S, Serke M, Brudler O. Lymphocyte activation by phytohaemagglutinin and pokeweed mitogen. Identification of proliferating cells by monoclonal antibodies. *J Immunol Methods* 1987, 99: 167-172 [PMID: 3108406 DOI: 10.1016/0022-1759(87)90122-0]

46 Dye JR, Palvanov A, Guo B, Rothstein TL. B cell receptor cross-talk: exposure to lipopolysaccharide induces an alternate pathway for B cell receptor-induced ERK phosphorylation and NF-kappa B activation. *J Immunol* 2007; 179: 229-235 [PMID: 17579042]

47 Wade WF. B-cell responses to lipopolysaccharide epitopes: Who sees what first - does it matter? *Am J Reprod Immunol* 2006; 56: 329-336 [PMID: 17076677 DOI: 10.1111/j.1600-0897.2006.00433.x]

48 Dubois V, Lambeir AM, Van der Veken P, Augustyns K, Creemers J, Chen X, Scharpé S, De Meester S. Fibroblast activation protein and chronic liver disease. *Clin Lab Immunol* 2005; 112: 269-277 [PMID: 15804855 DOI: 10.1016/j.jlb.0906546]

49 Yarden Y, Slivkovski MX. Untangling the ErbB signaling network. *Nat Rev Mol Cell Biol* 2001; 2: 127-137 [PMID: 11252954 DOI: 10.1038/35052073]

50 Maes MB, Dubois V, Brandt I, Lambeir AM, Van der Veken P, Augustyns K, Cheng JG, Chen X, Scharpé S, De Meester I. Dipeptidyl peptidase 8/9-like activity in human leucocytes. *J Leukoc Biol* 2007; 81: 1252-1257 [PMID: 17287297 DOI: 10.1189/jlb.0906546]

51 Pirina F, Schneider E, Betticher DC, Borch MM. Mitomycin C induces apoptosis and caspase-8 and -9 processing through a caspase-3 and Fas-independent pathway. *Cell Death Differ* 2002; 9: 905-914 [PMID: 12181741 DOI: 10.1038/sj.jid.4401062]

52 Kloner RA. No reflow revisited. *J Am Coll Cardiol* 1989; 14: 1814-1815 [PMID: 2584573 DOI: 10.1016/s0735-1097(99)01486-3]

53 Wang XM, Yao TW, Nadii NA, Osborne B, McLaughan GW, Gorrell MD. Fibroblast activation protein and chronic liver disease. *Front Biosci* 2008; 13: 3168-3180 [PMID: 17981786 DOI: 10.2741/2949]

54 De Meester I, Corom Y, Van Damme J, Scharpé S. CD26, let it cut or cut it down. *Immunol Today* 1999; 20: 367-375 [PMID: 10431157 DOI: 10.1016/s0167-5699(99)01486-3]

55 Novobrantseva TV, Majeau GR, Amatucci A, Kogan S, Brenner I, Cosala S, Slhombick MJ, Koteliantsky V, Hochman PS, Ibraghimov A. Attenuated liver fibrosis in the absence of B cell receptor activation by pokeweed mitogen induces production of TNF-alpha and GM-CSF and helper signaling by IL-1 and IL-6 results in IL-2-dependent T cell growth. *Eur J Immunol* 1993; 4: 269-277 [PMID: 8266417]