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Regulation of Major Histocompatibility Complex Class I Molecule Expression on Cancer Cells by Amyloid Precursor-Like Protein 2

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Abstract

The three members of the amyloid precursor protein family in mammals (amyloid precursor protein, amyloid precursor-like protein-1, and amyloid precursor-like-protein-2 (APLP2)) have been implicated in a large array of intracellular processes, which include development, transcription, apoptosis, metabolism, and the cell cycle. A series of studies by our laboratories has

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demonstrated that APLP2 is highly expressed by many cancer cell lines (with the highest expression in pancreatic cancer cell lines) and that it facilitates major histocompatibility complex (MHC) class I molecule endocytosis. This review focuses on this recently revealed function of APLP2 relevant to tumor immunology: that it acts as a novel regulator of MHC class I molecule surface expression.

Keywords

amyloid precursor-like protein 2; antigen presentation; cancer; endocytosis; major histocompatibility complex class I molecule; human leukocyte antigen; tumor antigen; tumor immunology; trafficking

Amyloid precursor-like protein 2

In the amyloid precursor protein (APP) family in mammals, there are three members: amyloid precursor protein (APP), amyloid precursor-like protein-1 (APLP1), and amyloid precursor-like-protein-2. APP, containing the pathogenic beta-amyloid domain implicated in Alzheimer's disease, is the most studied of the three, but there is an increasing amount of knowledge about the functions of the other two members, particularly APLP2. There is growing understanding that at least two of the members of this family, APP and APLP2, have multiple functions that potentially influence the biology of tumor cells. For example, APLP2 is involved in cellular functions as diverse as signaling, adhesion, migration, and mitosis [1–6]. Although APLP1 expression is primarily limited to the nervous system, APP and APLP2 are ubiquitously expressed [7–8]. The APP, APLP1, and APLP2 proteins all share a high degree of sequence similarity; however, the region of APP most implicated in Alzheimer's disease (the amyloid peptide) is not shared with APLP1 and APLP2 [9]. Like APP, APLP2 exists as several isoforms of a type I transmembrane protein that is glycosylated and phosphorylated (Figure 1), and both proteins undergo complex cleavages by proteolytic enzymes (Figure 2) [6–7, 9–11]. Sites of APLP2 cleavage by ADAM10 (an alpha-secretase), BACE1 (a beta-secretase), and gamma-secretase have been identified [11]. Portions of APLP2 responsible for its intracellular signaling functions include cleaved C-terminal fragments [9–10].

Several studies have shown over-expression of APP in human cancer tissue (pancreatic, thyroid, prostate, colon, and oral squamous cell cancer tissues) [12–17]. In prostate and oral squamous cell carcinoma, higher APP expression in tumors correlates with a worse survival rate [15, 17]. APP secreted by prostate and pancreatic cancer cell lines increases proliferation by binding to an unidentified cell-surface receptor, and treatment of prostate tumors with APP siRNA reduces tumor growth [12–13, 17]. Furthermore, treatment of pancreatic and colon cancer cells with the histone deacetylase inhibitor valproic acid inhibits proliferation by down-regulating the secretion of APP [12]. In addition to secretion by pancreatic cancer cell lines, APP is secreted by human pancreatic tumors [12–13, 18]. APLP2 is elevated in tumors [19] and increased in invasive breast cancer compared to ductal carcinoma *in situ* [20]. Like APP, APLP2 is also secreted by a pancreatic cancer cell line [21]. In our own studies of cancer cell lines, we have analyzed the levels of expression of APLP2 by several human cell lines. We found especially high levels of APLP2 in the pancreatic tumor cell lines S2-013, SUIT2 and Hs766T, as well as in the prostate cancer cell line DU145 [22]. APLP2 was expressed at a slightly lower level by MDA-MB435S (formerly classified as a breast cancer cell line, but currently reclassified as a melanoma line), and expressed at a moderate level by the HeLa cell line [22]. In contrast, lymphoma cell lines (SU-DHL-6 and CL-01) had low levels of APLP2 [22]. Thus, there was a range among the cell lines in regard to APLP2 expression, with most lines expressing moderate to

high levels, and with the pancreatic cancer cell line SUIT2 and its subclone S2-013 expressing the highest levels among the cell lines tested.

MHC class I molecule

MHC class I molecule presentation of tumor peptides is necessary for the recognition and killing of cancer cells by T lymphocytes. Binding of the MHC class I heavy chain to a peptide antigen takes place in the endoplasmic reticulum, in a process that requires participation of several chaperone proteins [23–27]. Several reports have also suggested that MHC class I trafficking between the endoplasmic reticulum and the plasma membrane does not occur simply by bulk flow, but is instead a regulated process [24, 28–33]. MHC class I molecules at the cell surface are internalized and then recycled [34–35]. The process of internalization of MHC class I molecules is dependent on an amino acid sequence located in the MHC class I molecule cytoplasmic tail [36]. Some studies have shown that MHC class I molecule endocytosis occurs by a clathrin-mediated mechanism [37]; however, other studies have suggested a clathrin-independent mechanism instead [38]. The recycling of MHC class I molecules involves the Eps15 homology domain-containing protein (EHD) 1, located on intracellular tubules [35, 39], as well as the closely related EHD4 protein [40].

Co-localization of APLP2 with MHC class I molecules in cancer cell lines

In S2-013 pancreatic cancer cells that were permeabilized and stained with fluorescently labeled antibodies for APLP2 and MHC class I molecules, extensive co-localization of MHC class I molecules with APLP2 in cytoplasmic vesicles was revealed [22]. By comparison, very little colocalization of APLP2 with internalized transferrin receptor could be detected in S2-013 cells [22]. Many of the vesicles in which APLP2/MHC co-localization occurred in S2-013 cells were early endosomes, as indicated by the presence of EEA1 and Rab5, although some were recycling endosomes, as indicated by Rab11 staining [22]. Likewise, in HeLa cells, endocytosed MHC class I molecules were found in association with APLP2 in Rab5-positive early endosomes, as well as in the Golgi complex [22, 41]. To ascertain whether APLP2 co-localized with MHC class I after the APLP2 and MHC class I molecules had been internalized from the cell surface, both proteins were antibody-labeled at the cell surface and were subsequently found to be co-localized in the same early endosomes [42].

Binding of MHC class I molecules to APLP2 in cancer cell lines

S2-013 pancreatic cancer cells express HLA-A*0206 and HLA-A*2402, and MDA-MB435S melanoma cells express HLA-A*2402. In these cell lines, APLP2 was found to bind to HLA-A24, and even more strongly to HLA-A2 [22]. By comparison, very little binding of the transferrin receptor to APLP2 was detectable, indicating specificity in APLP2's interactions [22]. In several types of cell lines, including cervical cancer and melanoma cell lines, APLP2 was demonstrated to bind strongly to the mouse MHC class I molecule K^d [41, 43–46]. APLP2 also binds, with a range of affinities, to other murine MHC class I molecules besides K^d [46]. The presence of the MHC class I light chain, β_2 -microglobulin, is needed for K^d/APLP2 binding [45], and APLP2 cannot bind well to K^d molecules having open, peptide-free binding grooves [44]. Mutational studies have shown that the conserved (α 3/transmembrane/cytoplasmic) membrane-proximal region and the polymorphic (α 1/ α 2) membrane-distal regions of the MHC class I molecule are both involved in interaction with APLP2 [46].

APLP2 is bound to MHC molecules that have been endocytosed, as demonstrated by surface labeling of MHC class I molecules, warming the cells to allow internalization of the MHC molecules, isolation of the labeled MHC class I molecules, and immunoblotting for APLP2 [41]. Furthermore, to assess if APLP2 and MHC class I bind to each other at the cell surface

even before their internalization, MHC class I molecules at the surface of HeLa cells were antibody labeled on ice to prevent internalization, and immunoblots of electrophoresed lysates revealed co-immunoprecipitated APLP2 [42]. Thus, the APLP2 and MHC class I molecules bind to each other at the cell surface, and endocytosed MHC class I molecules are bound to APLP2 [41–42].

The effect of APLP2 on the surface expression of MHC class I molecules on cancer cell lines

To examine whether APLP2 influences the expression of MHC class I molecules at the surface of a cancer cell line, APLP2's impact on the expression of HLA-A24 was investigated [22]. Increasing the expression of APLP2 further by APLP2 transfection of MDA-MB435S melanoma cells caused a reduction in surface expression of the HLA-A24 molecule, as well as a decrease in the total cell-surface MHC class I expression. With the cervical cancer line HeLa, the effect of APLP2 on the surface expression of transfected HLA-A24 was investigated by the use of confocal microscopy, which showed that the HeLa cells transfected with additional APLP2 had a perceptible decrease in the amount of HLA-A*2402 in the vicinity of the plasma membrane. Elevated expression of APLP2 also decreased surface expression of the mouse MHC class I molecule K^d ectopically expressed in HeLa cells (to ~60% of the original level), and the opposite was also true, in that APLP2-specific shRNA raised the surface expression of K^d (~1.7-fold) on HeLa cells [41, 44, 46]. Expression of elevated levels of APLP2 did not induce cellular stress (as shown by the lack of change in GrP94 and BiP expression), and thus the effects of APLP2 on MHC class I expression are not attributable to a non-specific cell stress reaction [41]. Furthermore, surface MHC class I surface expression was not decreased when an irrelevant protein (transferrin receptor) was over-expressed in HeLa cells [41].

The effect of APLP2 on MHC class I molecule endocytosis in cancer cell lines

In confocal microscopy studies it was observed that increased expression of APLP2 resulted in more MHC class I molecule endocytosis [41]. Since the mechanisms underlying the endocytosis of APLP2 itself had not been previously established, we examined APLP2 internalization in HeLa cells. The HeLa cells were transfected with the dominant-negative GFP-dynamin II K44A mutant or the C-terminal fragment of AP180-FLAG to test whether APLP2 is endocytosed by a clathrin-mediated route, and both did prove to block APLP2 endocytosis. On the other hand, consistent with the reports that MHC class I molecule internalization is primarily clathrin-independent [38, 47], MHC class I endocytosis remained relatively unaffected. The ability of APLP2 to re-channel MHC class I molecules into the clathrin-mediated endocytosis pathway may be the source of some of the diversity in the literature about the route of MHC class I internalization [37–38]. Furthermore, we found that blocking clathrin-dependent endocytosis prevented APLP2 from facilitating MHC class I molecule endocytosis, suggesting that APLP2 must be endocytosed for it to increase MHC class I internalization.

In HeLa cells over-expressing APLP2, the majority of the internalized antibody-tagged MHC class I molecules were retained within the cell for a prolonged time, suggesting that APLP2 reduces the recycling of MHC class I molecules [42]. To determine the localization of the MHC class I molecules that were not recycled, the HeLa cells over-expressing APLP2 were treated with ammonium chloride to inhibit lysosome function, revealing that the internalized MHC class I molecules had accumulated within the lysosomes. Therefore, the APLP2-associated endocytosis of MHC class I molecules leads to their removal from the

cell surface and subsequent destruction, rather than their recycling and re-emergence at the cell surface (Figure 3). The elevated APLP2 expression in cancer cell lines can thus enhance the reduction of MHC class I present these cells, and serve as a means of immune editing. As evidence that this regulation of MHC class I surface expression by APLP2 might also occur *in vivo*, we have found that the level of APLP2 is increased in human pancreatic adenocarcinoma tissue samples, relative to normal pancreatic tissue samples (H. Peters et al., unpublished data).

APLP2 domains: roles in APLP2's effect on MHC class I internalization

Within the APLP2 cytoplasmic tail in both human and mouse, the sequence NPTYKYL contains two endocytic motifs known to bind to the AP μ subunits of AP-2 complexes [48, 49]. Mutation of the tyrosine residue right in the middle of this sequence did not disrupt binding of the MHC class I molecule to APLP2, but did inhibit endocytosis of APLP2, as well as its ability to facilitate MHC class I endocytosis [42]. Consistent with the effect of this mutation on APLP2 endocytosis, transfection of HeLa with AP-2 μ 2 siRNA largely blocked APLP2 internalization [42].

Although our studies have shown a role for the NPTYKYL C-terminal sequence in APLP2's effect on MHC class I internalization, many questions remain regarding the mechanisms of APLP2's activity. One question is the role of the full-length, transmembrane form of APLP2 versus the various APLP2 cleavage fragments, including the C-terminal fragments that contain the NPTYKYL sequence. We have demonstrated that full-length APLP2 expressed in cancer cell lines binds to the MHC class I molecule [22], but have also found that C-terminal fragments of APLP2 associate with MHC class I as well (H. Peters, A. Tuli, et al., unpublished data). Whether these APLP2 fragments play an active role (either a potentiating role or blocking role) in APLP2's facilitation of MHC class I molecule endocytosis is currently under examination.

Furthermore, it is unknown whether the secreted N-terminal fragment of APLP2 also binds to the MHC class I molecule at the surface of tumor cells and influences its surface expression or its recognition by T cell receptors. Our observation that the polymorphic membrane-distal, as well as the conserved membrane-proximal, regions of the MHC class I molecule interact with APLP2 raise that possibility [46]. Our future collaborative studies can utilize isolated fractions of concentrated pancreatic cancer cell culture medium, shown by one of our laboratories (the MacDonald laboratory) to contain the secreted N-terminus of APLP2, to assess whether the N-terminal ectodomain of APLP2, in addition to sequences in the APLP2 C-terminus, affects the localization and function of MHC class I molecules.

Although there have been extensive studies of MHC class I molecule assembly, the exploration of the internalization, recycling, and degradation of MHC class I molecules has been much more limited. Nevertheless, both processes control the extent of antigen presentation by MHC class I molecules to T lymphocytes. Studies such as these are necessary to be able to understand, and to exploit in therapeutic strategies, the full array of mechanisms by which MHC class I cell-surface expression on tumor cells is regulated.

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References

1. Cappai R, Mok SS, Galatis D, et al. Recombinant human amyloid precursor-like protein 2 (APLP2) expressed in the yeast *Pichia pastoris* can stimulate neurite outgrowth. *FEBS Letters*. 1999; 442:95–8. [PubMed: 9923612]
2. Guo J, Thinakaran G, Guo Y, Sisodia SS, Yu FX. A role for amyloid precursor-like protein 2 in corneal epithelial wound healing. *Investig Ophthalmol Vis Sci*. 1998; 39:292–300. [PubMed: 9477985]
3. Li XF, Thinakaran G, Sisodia SS, Yu FS. Amyloid precursor-like protein 2 promotes cell migration toward fibronectin and collagen IV. *J Biol Chem*. 1999; 274:27249–56. [PubMed: 10480944]
4. Rassoulzadegan M, Yang Y, Cuzin F. APLP2, a member of the Alzheimer precursor protein family, is required for correct genomic segregation in dividing mouse cells. *EMBO J*. 1998; 17:4647–56. [PubMed: 9707424]
5. Thinakaran G, Kitt CA, Roskams AJ, Slunt HH, Masliah E, von Koch C, Ginsberg SD, Ronnett GV, Reed RR, Price DL. Distribution of an APP homolog, APLP2, in the mouse olfactory system: a potential role for APLP2 in axogenesis. *J Neurosci*. 1995; 15:6314–26. [PubMed: 7472397]
6. Walsh DM, Minogue AM, Frigerio CS, Fadeeva JV, Wasco W, Selkoe DJ. The APP family of proteins: similarities and differences. *Biochem Soc Trans*. 2007; 35:416–20. [PubMed: 17371289]
7. Slunt HH, Thinakaran G, Von Koch C, Lo AC, Tanzi RE, Sisodia SS. Expression of a ubiquitous, cross-reactive homologue of the mouse beta-amyloid precursor protein (APP). *J Biol Chem*. 1994; 269:2637–44. [PubMed: 8300594]
8. Kim TW, Wu K, Xu JL, et al. Selective localization of amyloid precursor-like protein 1 in the cerebral cortex postsynaptic density. *Brain Res Mol Brain Res*. 1995; 32:36–44. [PubMed: 7494461]
9. Zheng H, Koo EH. The amyloid precursor protein: beyond amyloid. *Mol Neurodegen*. 2006; 1:e5.
10. Jacobsen KT, Iverfeldt K. Amyloid precursor protein and its homologues: a family of proteolysis-dependent receptors. *Cell Mol Life Sci*. 2009; 66:2299–318. [PubMed: 19333550]
11. Höggl S, Kuhn PH, Colombo A, Lichtenthaler SF. Determination of the proteolytic cleavage sites of the amyloid precursor-like protein 2 by the proteases ADAM10, BACE1 and γ -secretase. *PLoS One*. 2011; 6:e21337. [PubMed: 21695060]
12. Venkataramani V, Rossner C, Iffland L, et al. Histone deacetylase inhibitor valproic acid inhibits cancer cell proliferation via down-regulation of the Alzheimer amyloid precursor protein. *J Biol Chem*. 2010; 285:10678–89. [PubMed: 20145244]
13. Hansel DE, Rahman A, Wehner S, Herzog V, Yeo CJ, Maitra A. Increased expression and processing of the Alzheimer amyloid precursor protein in pancreatic cancer may influence cellular proliferation. *Cancer Res*. 2003; 63:7032–7. [PubMed: 14612490]
14. Krause K, Karger S, Sheu S-Y, et al. Evidence for a role of the amyloid precursor protein in thyroid carcinogenesis. *J Endocrinol*. 2008; 198:291–9. [PubMed: 18480379]
15. Ko S-Y, Lin S-C, Chang K-W, et al. Increased expression of amyloid precursor protein in oral squamous cell carcinoma. *Int J Cancer*. 2004; 111:727–32. [PubMed: 15252842]
16. Ko S-Y, Chang K-W, Lin S-C, Hsu H-C, Liu T-Y. The repressive effect of green tea ingredients on amyloid precursor protein (APP) expression in oral carcinoma cells in vitro and in vivo. *Cancer Lett*. 2007; 245:81–9. [PubMed: 16458426]
17. Takayama K, Tsutsumi S, Suzuki T, et al. Amyloid precursor protein is a primary androgen target gene that promotes prostate cancer growth. *Cancer Res*. 2009; 69:137–42. [PubMed: 19117996]
18. Gronborg M, Kristiansen TZ, Iwahori A, et al. Biomarker discovery from pancreatic cancer secretome using a differential proteomic approach. *Mol Cell Proteomics*. 2006; 5:157–71. [PubMed: 16215274]

19. Covell DG, Wallqvist A, Rabow AA, Thanki N. Molecular classification of cancer: unsupervised self-organizing map analysis of gene expression microarray data. *Mol Cancer Therap.* 2003; 2:317–32. [PubMed: 12657727]
20. Abba MC, Drake JA, Hawkins KA, et al. Transcriptomic changes in human breast cancer progression as determined by serial analysis of gene expression. *Breast Cancer Res.* 2004; 6:499–513.
21. Mauri P, Scarpa A, Nascimbeni AC, et al. Identification of proteins released by pancreatic cancer cells by multidimensional protein identification: a strategy for identification of novel cancer markers. *FASEB J.* 2005; 19:1125–7. [PubMed: 15985535]
22. Tuli A, Sharma M, Wang X, et al. Amyloid precursor-like protein 2 association with HLA class I molecules. *Cancer Immunol Immunother.* 2009; 58:1419–31. [PubMed: 19184004]
23. Cresswell P, Bangia N, Dick T, Diedrich G. The nature of the MHC class I peptide loading complex. *Immunol Rev.* 1999; 172:21–8. [PubMed: 10631934]
24. Paquet ME, Cohen-Doyle M, Shore GC, Williams DB. Bap29/31 influences the intracellular traffic of MHC class I molecules. *J Immunol.* 2004; 172:7548–55. [PubMed: 15187134]
25. Park B, Lee S, Kim E, et al. Redox regulation facilitates optimal peptide selection by MHC class I during antigen processing. *Cell.* 2006; 127:369–82. [PubMed: 17055437]
26. Serwold T, Gonzalez F, Kim J, Jacan R, Shastri N. ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum. *Nature.* 2002; 419:480–3. [PubMed: 12368856]
27. Saric T, Chang SC, Hattori A, et al. An IFN- γ -induced aminopeptidase in the ER, ERAP1, trims precursors to MHC class I-presented peptides. *Nat Immunol.* 2002; 3:1169–76. [PubMed: 12436109]
28. Joyce S. Traffic control of completely assembled MHC class I molecules beyond the endoplasmic reticulum. *J Mol Biol.* 1997; 266:993–1001. [PubMed: 9086276]
29. Marguet D, Spiliotis ET, Pentcheva T, Lebowitz M, Schneck J, Edidin M. Lateral diffusion of GFP-tagged H2L^d molecules and of GFP-TAP1 reports on the assembly and retention of these molecules in the endoplasmic reticulum. *Immunity.* 1999; 11:231–40. [PubMed: 10485658]
30. Spiliotis ET, Manley H, Osorio M, Zuniga MC, Edidin M. Selective export of MHC class I molecules from the ER after their dissociation from TAP. *Immunity.* 2000; 13:841–51. [PubMed: 11163199]
31. Sohn HW, Shin YK, Lee IS, et al. CD99 regulates the transport of MHC class I molecules from the Golgi complex to the cell surface. *J Immunol.* 2001; 166:787–94. [PubMed: 11145651]
32. Pencheva T, Edidin M. Clustering of peptide-loaded MHC class I molecules for endoplasmic reticulum export imaged by fluorescence resonance energy transfer. *J Immunol.* 2001; 166:6625–32. [PubMed: 11359816]
33. Paulsson KM, Kleijmeer MJ, Griffith J, et al. Association of tapasin and COPI provides a mechanism for the retrograde transport of major histocompatibility complex (MHC) class I molecules from the Golgi complex to the endoplasmic reticulum. *J Biol Chem.* 2002; 277:18266–71. [PubMed: 11884415]
34. Reid PA, Watts C. Cycling of cell-surface MHC glycoproteins through primaquine-sensitive intracellular compartments. *Nature.* 1990; 346:655–7. [PubMed: 2166918]
35. Caplan S, Naslavsky N, Hartnell LM, et al. A tubular EHD1-containing compartment involved in the recycling of major histocompatibility complex class I molecules to the plasma membrane. *EMBO J.* 2002; 21:2557–67. [PubMed: 12032069]
36. Vega MA, Strominger JL. Constitutive endocytosis of HLA class I antigens requires a specific portion of the intracytoplasmic tail that shares structural features with other endocytosed molecules. *Proc Natl Acad Sci USA.* 1989; 86:2688–92. [PubMed: 2495533]
37. Dasgupta JD, Watkins S, Slayter H, Yunis EJ. Receptor-like nature of class I HLA: endocytosis via coated pits. *J Immunol.* 1988; 141:2577–80. [PubMed: 2902138]
38. Radhakrishna H, Donaldson JG. ADP-ribosylation factor 6 regulates a novel plasma membrane recycling pathway. *J Cell Biol.* 1997; 139:49–61. [PubMed: 9314528]
39. Naslavsky N, Boehm M, Backlund PS Jr, Caplan S. Rabenosyn-5 and EHD1 interact and sequentially regulate protein recycling to the plasma membrane. *Mol Biol Cell.* 2004; 15:2410–22. [PubMed: 15020713]

40. Sharma M, Naslavsky N, Caplan S. A role for EHD4 in the regulation of early endosomal transport. *Traffic*. 2008; 9:995–1018. [PubMed: 18331452]
41. Tuli A, Sharma M, McIlhaney MM, Talmadge JE, Naslavsky N, Caplan S, Solheim JC. Amyloid precursor-like protein 2 increases the endocytosis, instability, and turnover of the H2-K^d MHC class I molecule. *J Immunol*. 2008; 181:1978–87. [PubMed: 18641335]
42. Tuli A, Sharma M, Capek HL, Naslavsky N, Caplan S, Solheim JC. Mechanism for amyloid precursor-like protein 2 enhancement of major histocompatibility complex class I molecule degradation. *J Biol Chem*. 2009; 284:34296–307. [PubMed: 19808674]
43. Feuerbach D, Burgert H-G. Novel proteins associated with MHC class I antigens in cells expressing adenovirus protein E3/19K. *EMBO J*. 1993; 12:3153–61. [PubMed: 8344254]
44. Morris CR, Petersen JL, Vargas SE, et al. The amyloid precursor-like protein 2 and the adenoviral E3/19K protein both bind to a conformational site on H-2K^d and regulate H-2K^d expression. *J Biol Chem*. 2003; 278:12618–23. [PubMed: 12506118]
45. Sester M, Feuerbach D, Frank R, Preckel T, Gutermann A, Burgert H-G. The amyloid precursor-like protein 2 associates with the major histocompatibility complex class I molecule K^d. *J Biol Chem*. 2000; 275:3645–54. [PubMed: 10652361]
46. Tuli A, Sharma M, Wang X, Naslavsky N, Caplan S, Solheim JC. Specificity of amyloid precursor-like protein 2 interactions with MHC class I molecules. *Immunogenetics*. 2008; 60(6): 303–13. [PubMed: 18452037]
47. Huet C, Ash JF, Singer SJ. The antibody-induced clustering and endocytosis of HLA antigens on cultured human fibroblasts. *Cell*. 1980; 21:429–38. [PubMed: 6996834]
48. Zhao X, Greener T, Al-Hasani H, Cushman SW, Eisenberg E, Greene LE. Expression of auxilin or AP180 inhibits endocytosis by mislocalizing clathrin: evidence for formation of nascent pits containing AP1 or AP2 but not clathrin. *J Cell Sci*. 2001; 114:353–65. [PubMed: 11148137]
49. Boll W, Rapoport I, Brunner C, Modis Y, Prehn S, Kirchhausen T. The mu2 subunit of the clathrin adaptor AP-2 binds to FDNPVY and YppØ sorting signals at distinct sites. *Traffic*. 2002; 3:590–600. [PubMed: 12121421]

Fig. 1.

A diagram of the APLP2 protein is shown, with the extracellular, transmembrane (TM) and cytoplasmic domains indicated. Subdomains of the protein that are also indicated on the diagram include the signal sequence, a region containing many aspartic acid and glutamic acid residues, and a Kunitz family protease inhibitor domain (which includes intramolecular disulfide bonds and an O-linked glycosylation site). Human APLP2 has been observed to undergo phosphorylation in the cytoplasmic domain at threonine 723, threonine 736, tyrosine 750, or tyrosine 755 (and a single report noted phosphorylation at tyrosine 757). The NPTYKYL sequence in the APLP2 cytoplasmic tail contains overlapping motifs for AP-2 binding. The tyrosine residue in the middle of the NPTYKYL sequence is tyrosine 755.

Fig. 2.

APLP2 cleavage is mediated by a series of secretase enzymes (α , β , γ , and ϵ secretases). Cleavage of APLP2 releases a large, secreted N-terminal domain, and produces a series of small fragments that include transmembrane and/or cytoplasmic domain sequences. In addition to the secretase cleavage sites, ADAM10 (not shown) cleaves APLP2 at amino acid position 670, which is located near the plasma membrane.

Fig. 3.

A model of APLP2's effect on MHC class I internalization is shown. MHC class I molecules, at the surfaces of cancer cells, present tumor antigens to cytotoxic T lymphocytes (not shown to scale) and induce lysis of the malignant cells. Cancer cells (especially pancreatic cancer cells over-express APLP2. APLP2 first interacts with the MHC class I molecule in the area of the plasma membrane. By clathrin-dependent endocytosis, APLP2 is internalized, and diverts the MHC class I molecule into the same pathway. In addition, APLP2 reduces MHC class I recycling by routing the internalized MHC class I molecules to lysosomes.