Modulation of Jagged1 processing by the E6 oncoprotein of Human Papillomavirus

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MOLECULAR CELL BIOLOGY

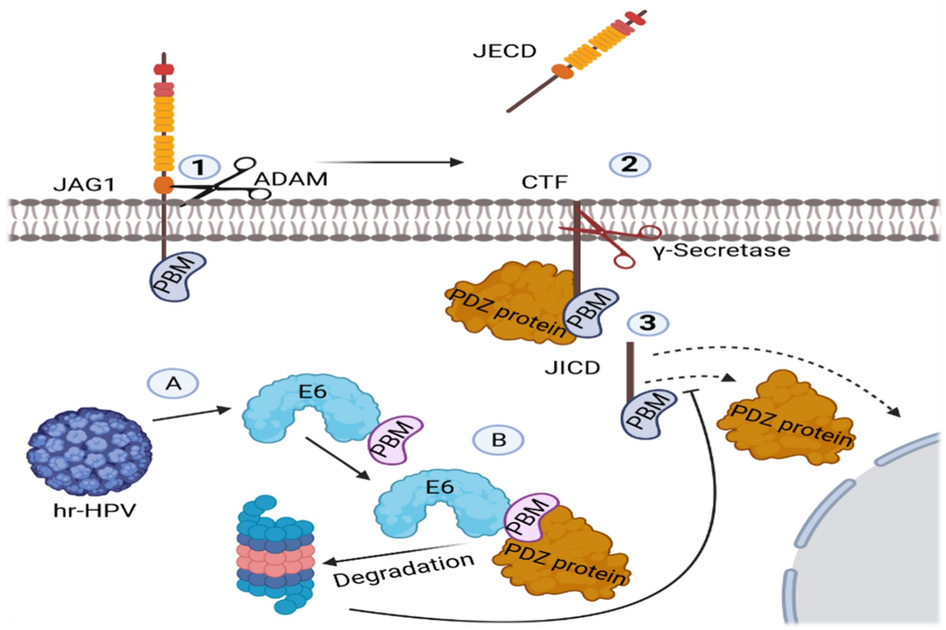


Figure 1. JAG1 processing and HPV infection. Extracellular cleaving of JAG1 by ADAM (1) removes the N-terminal domain of JAG1, leaving the C-terminal fragment (CTF) tethered to the cell membrane. The CTF is then cleaved (2) within its transmembrane domain, releasing the JICD, which has a terminal PBM. JICD interactions with PDZ-proteins are poorly understood (3). Hr-HPV encodes the E6 oncoprotein (A), which is able to bind and degrade hosts' PDZ-proteins (B).

Introduction

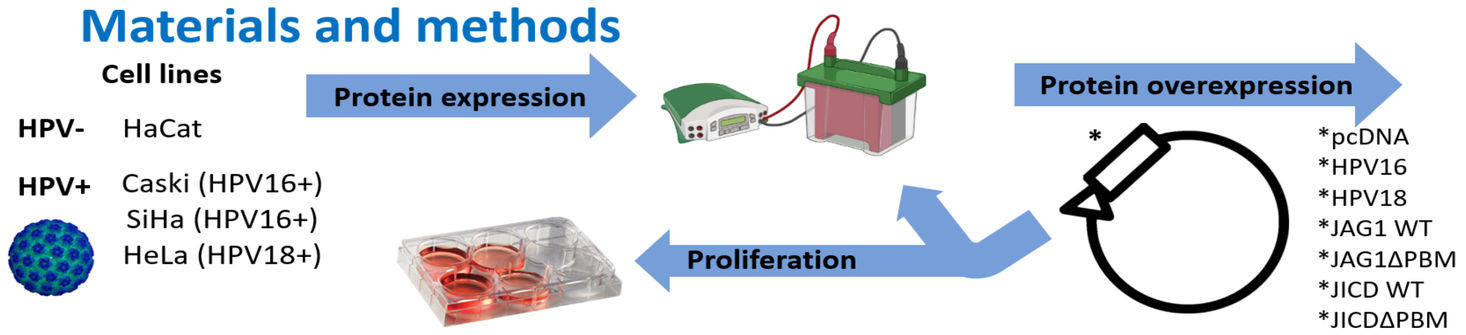
Around 95% of cervical cancer (CC) cases are caused by high-risk Human papillomaviruses (hr-HPVs). Hr-HPV encodes the E6 viral oncoprotein, which has a C-terminal PDZ (PSD-95, DLG-1 and ZO-1) binding motif (PBM), through which it binds to PDZ-proteins and promotes their proteosomal degradation[1]. The Notch pathway is a communication system with both oncogenic and suppressive functions in the context of HPV[1]. Notch activation is achieved by regulated intramembrane proteolysis (RIP), liberating the Notch intracellular domain (NICD) which translocates to the nucleus to modulate gene transcription. Interestingly, Jagged1 (JAG1) ligand also undergoes RIP, releasing the JAG1 intracellular domain (JICD) however, its fate is poorly described[2]. In addition, the C-terminal tail of JAG1 also encodes a PBM[3] giving it the ability to bind to PDZ-proteins.

Research questions

Is JAG1 processing modulated by hr-HPVs E6 oncoprotein?

Which is the function of JICD in HPV carcinogenesis?

Materials and methods



Results

Increase JAG1 expression by the E6 oncoprotein

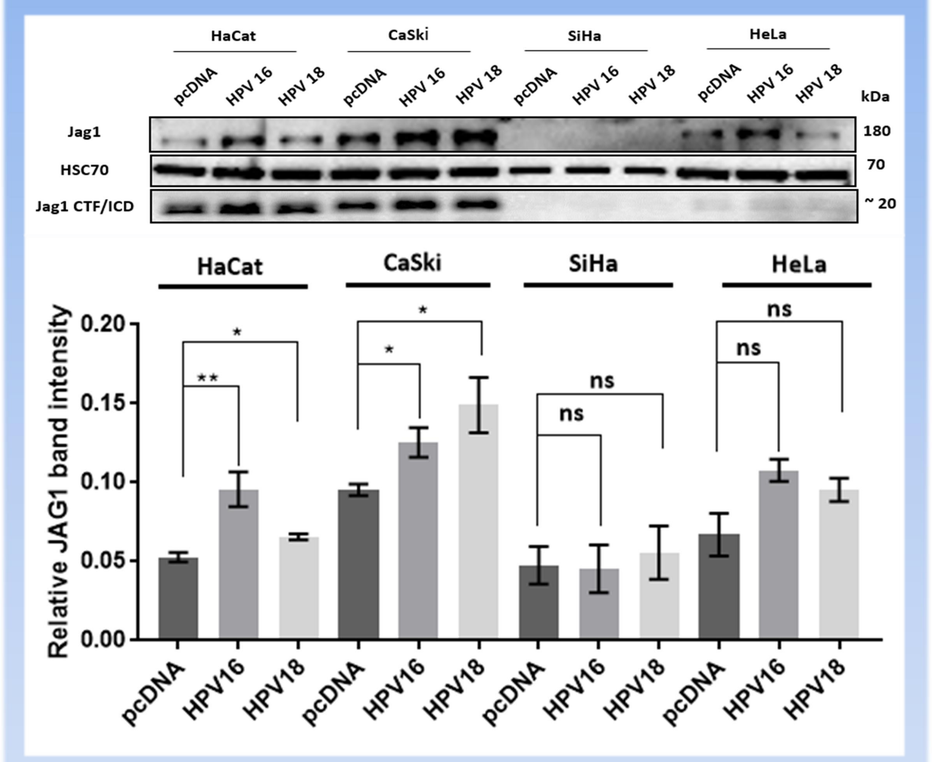


Figure 2. Modulation of JAG1 expression by the E6 oncoprotein. The E6 oncoprotein from HPV16 and HPV18 was overexpressed in immortalized keratinocytes (HaCat) and CC-derived cell lines. A statistically significant increase in JAG1 protein expression was observed in HaCat and CaSki cells after E6 overexpression (p=<0.005). The HeLa cells also showed an increase in JAG1 expression, although this wasn't statistically significant. SiHa cells showed the lowest expression of endogenous JAG1.

Modulation of JAG1 processing by E6 is PDZ-dependent

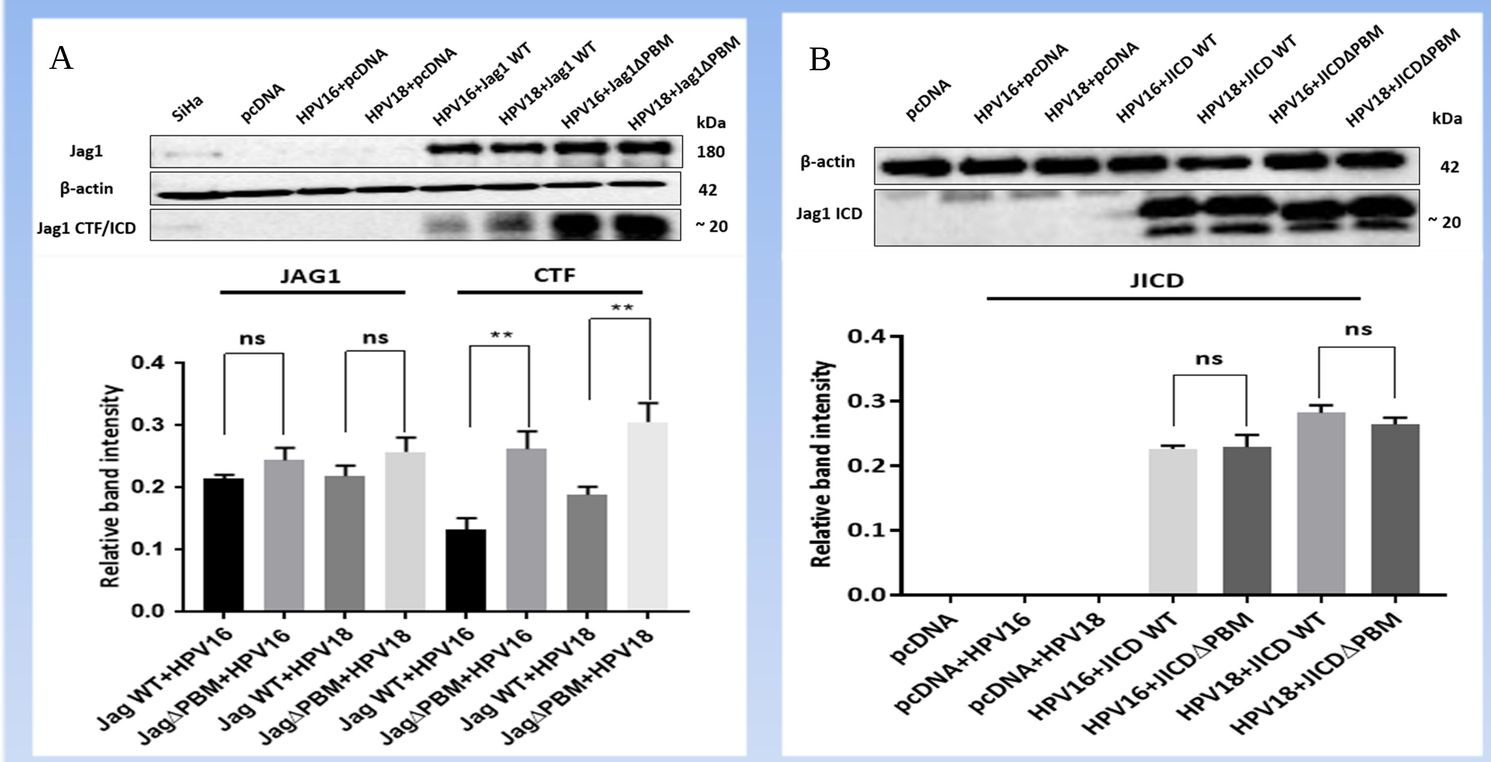
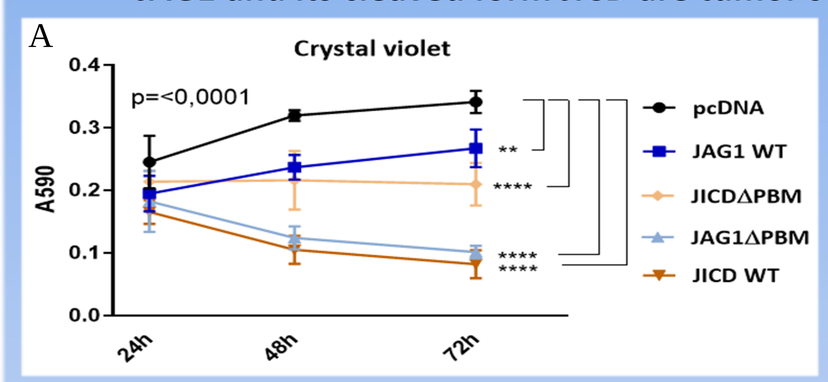


Figure 3. Modulation of JAG1 processing by the E6 oncoprotein. (A) JAG1-WT and mutant (JAG1 devoted to its C-terminal PBM, JAG1ΔPBM) were co-expressed with the E6 oncoprotein from HPV16 or HPV18 in SiHa cells. Statistically significant accumulation of CTF was observed after JAG1ΔPBM overexpression (p=<0.005). **(B)** JICD-WT and JICD mutant (JICDΔPBM) were co-expressed with the E6 oncoprotein of HPV16 and HPV18 in SiHa cells. There was no difference between JICD-WT and JICDΔPBM. These results suggest that the E6 oncoprotein promotes the cleaving of JAG1 full-length protein throughout a PDZ –dependent pathway.

JAG1 and its cleaved form JICD are tumor supressors in HPV carcinogenesis



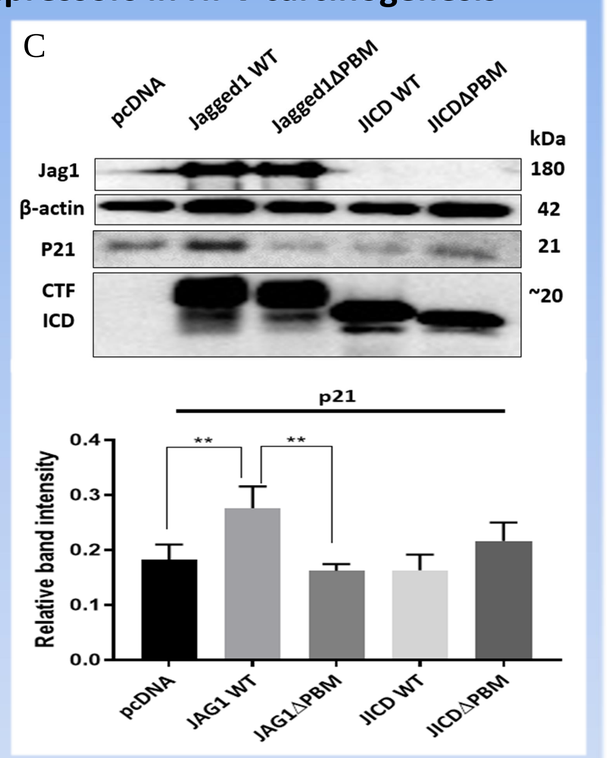


Figure 5. JAG1 and JICD inhibit cell proliferation through different pathways. The proliferation of Siha overexpressing the different JAG1 isoforms was evaluated by Crystal violet staining **(A)** and cell count **(B)**. Statistically significant suppression of proliferation was observed (p=<0.0001 and p=<0.0003, respectively). Statistical analysis was conducted using one-way ANOVA. **(C)** p21 expression in Siha cells overexpressing JAG1 isoforms. A significant increase in p21 expression was observed after JAG1-WT overexpression but not with JAG1ΔPBM. JICD has no effect on p21 expression, suggesting that JAG1-FL and its cleaved form JICD downmodulate proliferation through different pathways.

Conclusions

- **JAG1 processing is modulated by the E6 oncoprotein from hr-HPVs in a PDZ-dependent manner.**
- **JAG1 and JICD are tumor suppressors in HPV carcinogenesis.**
- **Decrease proliferation by JAG1 is p21 mediated and PDZ-dependent.**

Future perspectives

- **To determine how JAG1 processing affects the Notch pathway.**
- **To elucidate JAG1 interactions with PDZ proteins.**
- **To describe the tumor suppressor role of JICD.**

References

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