The role of AEG-1 in cervical cancer progression and response to therapy

Mireia Cartañá Seijo | Autonomous University of Barcelona | Biomedical Sciences 2012-2016

Universitat Autònoma de Barcelona

INTRODUCTION

- The **epithelial-mesenchymal transition** (**EMT**) converts epithelial cells into a mesenchymal phenotype, which is characterized by the loss of apicobasal polarity and cell-cell junctions, cytoskeleton remodelling and acquisition of migratory and invasive abilities.
- Having migrated to their target sites, mesenchymal cells may get their epithelial properties back through the reverse process termed **mesenchymal-epithelial transition** (**MET**).
- These processes essentially occur during morphogenesis and have been increasingly described in tissue repair and carcinogenesis during the last decade. Intriguingly, EMT and MET have been established as a crucial step in tumour progression, metastatic dissemination and therapy resistance (Fig.1).

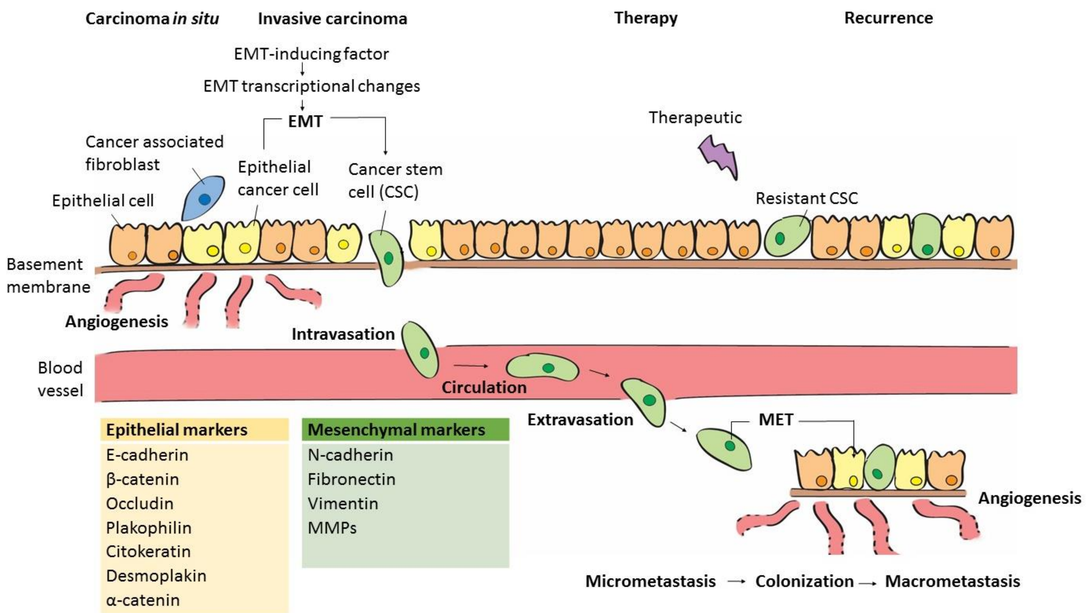


Figure 1. Role of EMT in tumour progression, metastasic dissemination, therapy resistance and recurrence.

- **Cervical cancer** arises from the narrow end of the uterus and is the second most prevalent female cancer worldwide.
- Its progression is accurately described by the Federation of Gynecology and Obstetrics (FIGO) guidelines. Despite the generally good prognosis at early stages, many patients are still being diagnosed at advanced stages (II-IV) where the mortality increases as a result of metastasis and recurrence (Fig. 2).
- **Cisplatin** (Platinol ®) and **paclitaxel** (Taxol ®) constitute first-line clinical chemotherapy in metastatic or recurrent cervical cancer. However, drug resistance limits the curative effect in some cases although the molecular mechanisms are not fully understood.

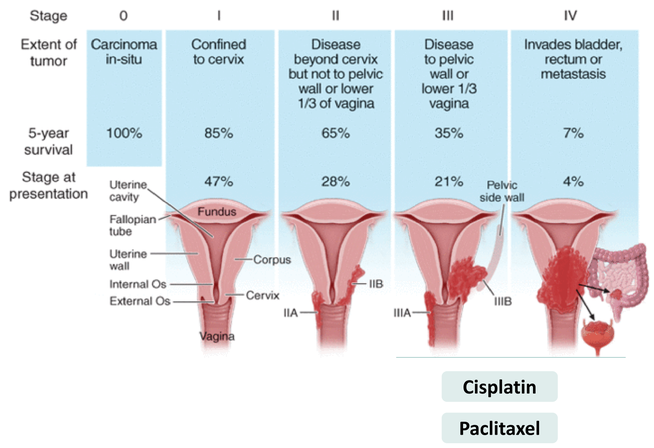


Figure 2. FIGO staging of cervical cancer, prognosis (*ie.* 5-year survival rate) and stage at presentation. Reproduced from Longo *et al.* (2015) [1].

- **Astrocyte elevated gene-1** (**AEG-1**), also known as metadherin (MTDH) or lysine-rich CEACAM-1-associated protein (LYRIC), is involved in physiological processes and has emerged in recent years as an important contributor to EMT. This correlates with tumour progression and chemoresistance in a variety of cancers.

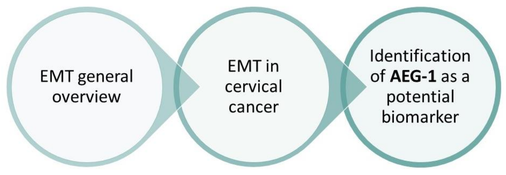
AIMS

By reviewing the current literature of EMT in the development of cervical cancer, this study aims to:

- Investigate the role of AEG-1 in tumour progression and response to therapy
- Evaluate the suitability of AEG-1 as a potential biomarker for cervical cancer monitoring and as a target for new drug development

METHODOLOGY

Primary research papers, case reports, theses and reviews were identified through systematic searches in PubMed database, mainly belonging to highly indexed journals and published between 2006-2016. Terms such as “EMT”, “cervical cancer”, “chemoresistance”, “metastasis”, “AEG-1”, “MTDH”, “LYRIC” were employed. A total of 51 were included in the final review.



RESULTS AND DISCUSSION

1. AEG-1 ACTS ON A PLETHORA OF SIGNALLING PATHWAYS

A wealth of evidence implicates AEG-1 in several signalling pathways (Fig. 3), namely:

- **Ha-Ras/PI3K/Akt**
- **MAPK**
- **NF-κB**
- **Wnt/β-catenin**

⇨ AEG-1 is implicated in pro-survival pathways

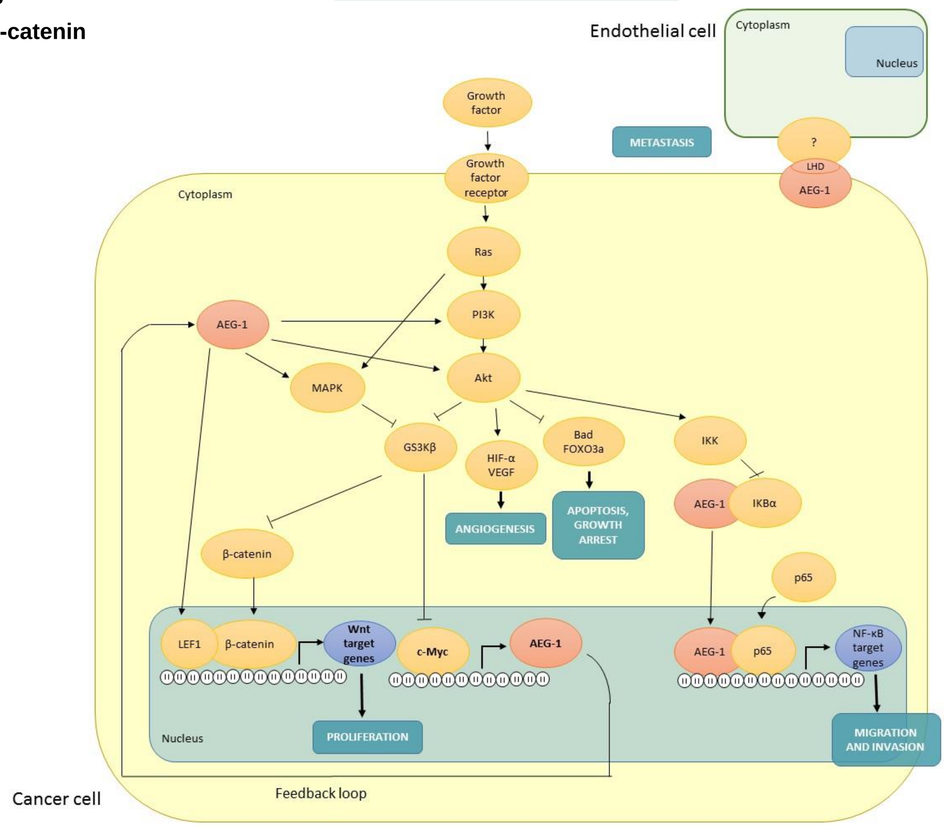


Figure 3. Hypothetical model of AEG1 molecular mechanisms in tumour progression and metastasis.

2. AEG-1 EXPRESSION IN CERVICAL CANCER AND CORRELATION WITH CLINICOPATHOLOGICAL FEATURES

AEG-1 is overexpressed in cervical cancer cell lines and human tumour samples within the cell membrane and cytoplasm, and significantly correlates with:

- FIGO staging (Fig. 4)
- Lymph node metastasis
- Overall survival (Fig. 5)

⇨ AEG-1 is suitable as an independent prognostic biomarker of disease outcome.

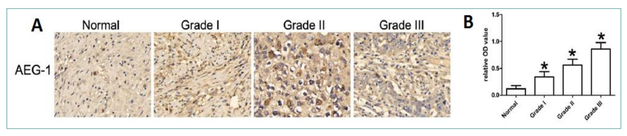


Figure 4. Immunohistochemical staining of AEG-1 in normal and cervical cancer tissue at different FIGO stages (**A**) and quantification of results expressed as relative OD value (**B**)**.** *p<0.001. Reproduced from Zhang *et al.* (2015) [2]

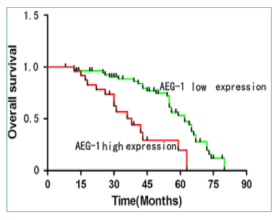


Figure 5. Kaplan-Meier analysis of overall survival according to AEG-1 expression. Reproduced from Huang *et al.* (2013) [3].

3. AEG-1 AND EMT

- **CCL20** activates AEG-1 via **MAPK** (Erk1/2) and **Akt** signaling pathways and increases the expression of mesenchymal markers **vimentin**, **N-cadherin**, and **MMP2** (Fig. 6) in cervical cancer cells *in vitro*.

⇩

AEG-1 contributes to EMT

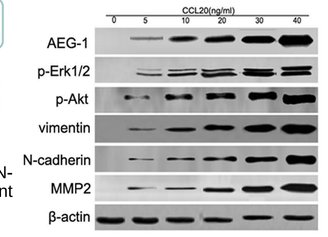


Figure 6. Western blot analysis of AEG-1, p-Erk1/2, p-Akt, vimentin, N-cadherin and MMP2 after increasing concentrations of CCL20 treatment in SiHa cells. Reproduced from Zhang *et al.* (2015) [2]

4. AEG-1 IN TUMOUR PROGRESSION AND METASTASIS

AEG-1 silencing in cervical cancer cells *in vitro* results in:

- Reduced **proliferation** (Fig. 7) and increased apoptosis
- Reduced **migration** and **invasion** (Fig. 8)
- Reduced **angiogenesis**

⇩

AEG-1 favours stem-cell like properties and promotes tumour progression and metastasis

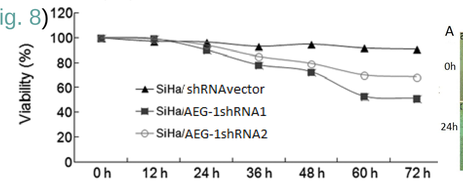


Figure 7. MTT cell proliferation assay of AEG-1 silencing in SiHa transfected cells. Reproduced from Long *et al.* (2013) [4].

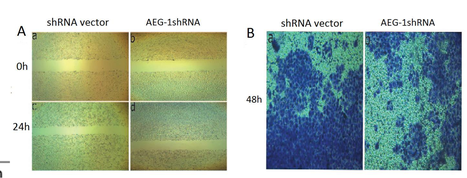


Figure 8. Scratch wound migration assay (**A**) and transwell invasion assay (**B**) in HeLa transfected cells. Reproduced from Liu *et al.* (2014) [5].

5. AEG-1 AND RESPONSE TO THERAPY

- AEG-1 promotes resistance to **cisplatin** and **paclitaxel** in cervical cancer cells *in vitro* (Fig. 9).
- AEG-1 induces:
  - Protective autophagy by upregulating **LC3**
  - Resistance to apoptosis by activating **MAPK** and **NF-κB**, thereby inhibiting the cleavage of **caspase-3**
- AEG-1 has been found to mediate chemoresistance via different mechanisms in various cancers (Fig. 10).

⇨ AEG-1 confers chemoresistance

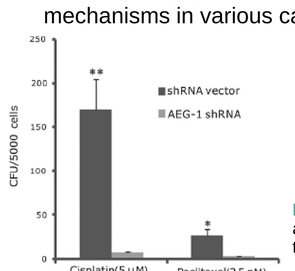


Figure 9. Colony formation units (CFU) in soft agar assay after cisplatin and paclitaxel treatment in HeLa transfected cells. Reproduced from Liu *et al.* (2014) [5].

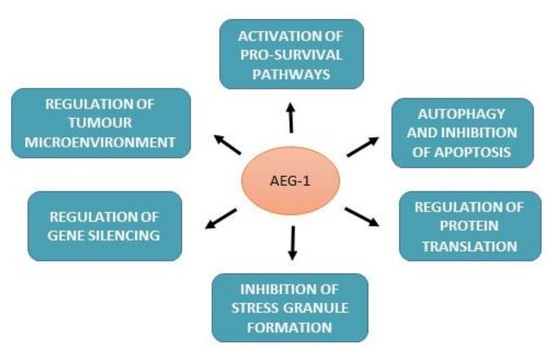


Figure 10. Mechanisms of AEG-1-induced chemoresistance.

CONCLUSIONS AND FUTURE APPROACHES

- EMT and MET are key players in regulating cellular plasticity in cancer, contributing to tumour progression, metastasis and resistance to therapy ⟶ inhibiting EMT represents an attractive therapeutic approach, but challenges include:
  - Complexity of signalling pathways
  - Block particular pathways to seek the highest effectiveness and minimal toxicity
- Cervical cancer metastasis and recurrence accounts for the majority of the related deaths and cannot be predicted due to the lack early-stage biomarkers.
- AEG-1 has been implicated in tumour progression and chemoresistance in several malignancies, including cervical cancer. However, a better understanding of the molecular mechanisms of the AEG-1-induced EMT still to be further elucidated.
- Yet, future research should be addressed in two main streams:
  - **Establishing AEG-1 as a reliable prognosis biomarker** by evaluating sensitivity and specificity
  - **Targeting AEG-1-induced EMT** to halt cervical cancer progression and chemoresistance. Possible strategies include:
    - Gene silencing via RNA interference technology (siRNAs and microRNAs)
    - Identifying functional domains in AEG-1 to develop novel small molecule inhibitors
    - Immunotherapy (anti-AEG-1 antibodies)

GLOSSARY

MMP:Matrix metalloproteinase **NF-κB**:Nuclear Factor-κ B **CDK2**:Cyclin-dependent kinase 2 **LHD**:Lung homing domain
PI3K:Phosphotidylinositol-3-kinase **CCL20**:Chemokine (C-C motif) ligand 20 **LC3**:Microtubule-associated protein 1A/1B-light chain 3

REFERENCES

[1]. Longo DL, Fauci AS, Kasper DL, Hauster SL, Jameson JL, *et al.* Harrison’s Principles of Internal Medicine. 18th Edition (2015). Available at: www.accessmedicine.com. Copyright © The McGraw-Hill Companies, Inc.
[2]. Zhang J, Zhu D, Lv Q, Yi Y, Li F, Zhang W. The key role of astrocyte elevated gene-1 in CCR6-induced EMT in cervical cancer. *Tumour Biol*. 2015; **36**(12): 9763-9767.
[3]. Huang K, Li LA, Meng Y, You Y, Fu X, Song L. High expression of astrocyte elevated gene-1 (AEG-1) is associated with progression of cervical intraepithelial neoplasia and unfavorable prognosis in cervical cancer. *World J Surg Oncol.* 2013; **11**: 297.
[4]. Long M, Dong K, Gao P, Wang X, Liu L et al. Overexpression of astrocyte-elevated gene-1 is associated with cervical carcinoma progression and angiogenesis. *Oncol Rep.* 2013; **30(3)**: 1414-1422.
[5]. Liu X, Wang D, Liu H, Feng Y, Zhu T et al. Knockdown of astrocyte elevated gene-1 (AEG-1) in cervical cancer cells decreases their invasiveness, epithelial to mesenchymal transition, and chemoresistance. *Cell cycle* 2014; 13(11): 1702–1707.