Differentiating cervical pre-cancer from invasive cancer from invasive cancer with the S5 DNA methylation classifier

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DIFFERENTIATING CERVICAL PRE-CANCER FROM INVASIVE CANCER WITH THE S5 DNA METHYLATION CLASSIFIER

16. Methylation

C. Banila [1], N. Belinda [1], M. Kleeman [1], C. Reuter [1], K. Cuschieri [2], G. Clifford [3], J. Cuzick [1], A. Lorincz [1]

[1]Queen Mary University of London - London (United kingdom), [2]University of Edinburgh - Edinburgh (United kingdom), [3]International Agency for Research on Cancer - Lyon (France)

Background / Objectives

Background: Persistent infection with high-risk human papillomavirus (hr-HPV) is an important co-factor in cervical cancer development and is associated with DNA methylation on both human and viral genes. The S5 DNA methylation classifier, based on target CpG sites of the human gene EPB41L3, and viral late gene regions of HPV16, HPV18, HPV31 and HPV33 (Lorincz A et al., 2016) has demonstrated better performance for detection of CIN2/3 women than either HPV16/18 genotyping, cytology or combination. We tested the performance of S5 in detecting invasive cancers versus pre-cancers and quantified the degree of separation between normal/CIN1, CIN2/3 and invasive cancer S5 scores.

Results

Methods: Methylation status of the S5 selected CpG sites was tested in DNA extracted from formalin-fixed biopsies from the Scottish HPV Archive (UK, n=24) and PreservCyt collected exfoliated cervical cell samples from the Scottish HPV Archive (UK, n=48) and the International Agency for Research on Cancer (Spain, n=100). Samples were histologically defined as negative/CIN1 (n=33), CIN2/3 (n=65) and invasive cancer (n=74). DNA bisulfite conversion was carried out and followed by pyrosequencing for the 6 components of S5. Average methylation was calculated for each marker to define the S5 score.

Conclusion

Results: Methylation at all sites increased proportionally with disease severity with a Cuzick trend value of z = 9.2933 ($p < 2.2x10-16$). The separation of normal/CIN1 from CIN2/3 and from cancer was highly significant (Mann Whitney test, both $p < 0.0001$). S5 also showed highly significant difference between CIN2/3 and invasive

cancer from both IARC-Spain ($p < 0.0001$) and Scottish ($p < 0.003$) cohorts. Receiver operating characteristic (ROC) curves were used to assess the diagnostic potential of S5 in differentiating cancers from CIN2/3. The area under the ROC curve (AUC) was 0.86 (CI 95%: 0.7965 to 0.9131, $p < 0.0001$) with a sensitivity of 79.8% and a specificity of 83.1%, based on a cut-off at highest Youden J index.

References

Conclusion: The S5 methylation classifier may be useful in cervical screening programs for differentiating pre-cancers from invasive cervical cancers in women infected with hr-HPV. Although the separation was very good, there is room for improvement in S5 by addition of new markers derived from an ongoing multi-omics study using next-Generation Sequencing.

References

Lorincz, A. T. et al. Validation of a DNA methylation HPV triage classifier in a screening sample. Int. J. cancer 138, 2745–51 (2016).