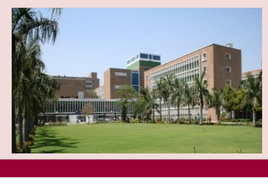
Prognostic importance of DNA repair gene polymorphisms in cervical cancer patients from India

Deepti Bajpai[1], Ayan Banerjee[1], Sujata Pathak[1], Sunesh Kumar Jain[2], Neeta Singh[1]

[1]Department of Biochemistry, [2]Department of Obstetrics and Gynecology

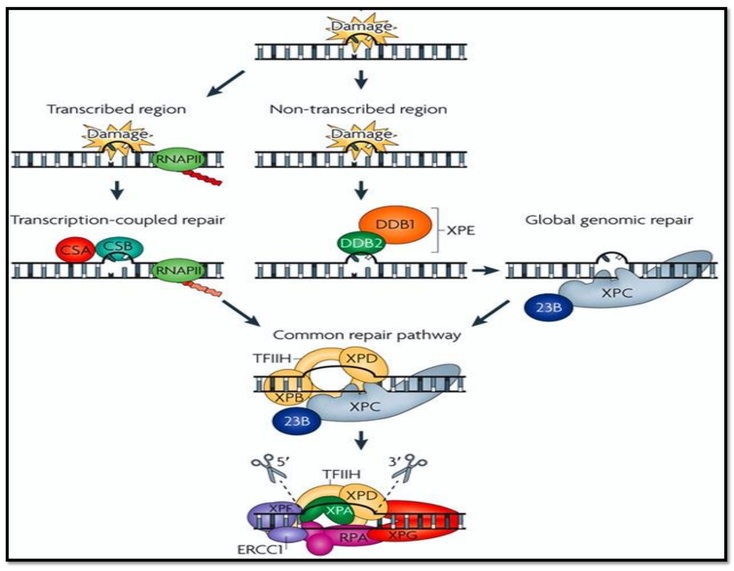
All India Institute of Medical Sciences, New Delhi, India



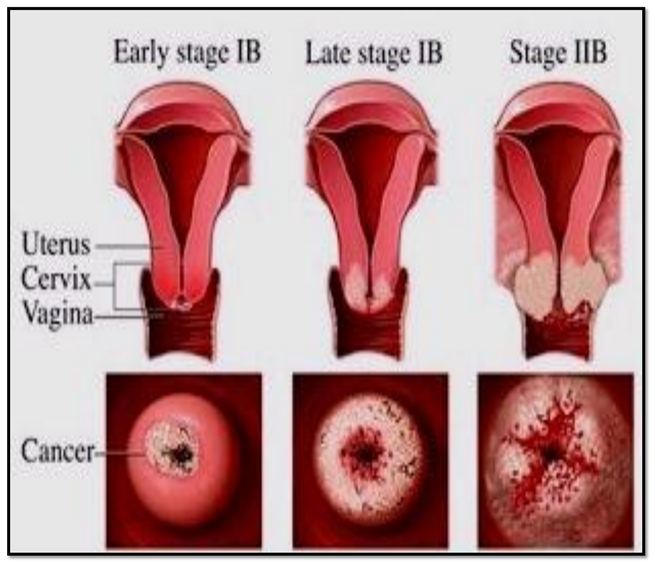
Introduction

- Cervical cancer is the fourth most common cancer in women, and the seventh overall, with an estimated 528,000 new cases (GLOBOCAN 2012).
- While it is well-known that carcinogenic human papillomaviruses (HPVs) are the causal agents of cervical cancer, HPV infections are extremely common relative to rare cancer incidence.
- Host genetic factors may play a role in cervical carcinogenesis and are thought to influence who develops persistent HPV infection and perhaps who further progresses to cancer.
- Many studies have now documented that the genes involved in DNA repair and maintenance of genome integrity are critically involved in protecting against mutations that lead to cancer and/or inherited genetic disease.
- Excision repair cross complementation group 2, or XPD is a protein involved in transcription coupled nucleotide excision repair.
- This protein is an essential part (subunit) of a group of proteins known as the general transcription factor IIH (TFIIH).
- Previous studies on the association between ERCC2 Lys751Gln and cervical cancer are inconclusive.
- Because a proper functioning of the ERCC2 gene is important for the genomic stability, its alterations may be associated with a higher cancer susceptibility.

Nucleotide Excission Repair Pathway



Cervical Cancer Pathology



Methodology

Cervical tissue and 5ml EDTA blood were collected from 65 healthy controls, 40 SIL and 65 cervical cancer patients coming to the Gynecology Dept. of AIIMS after informed consent

RNA was isolated from tissue samples using trizol reagent. Protein isolation was done using RIPA lysis buffer.

RNA level was quantified using Q-RT PCR.

Protein expession was checked using Western blotting.

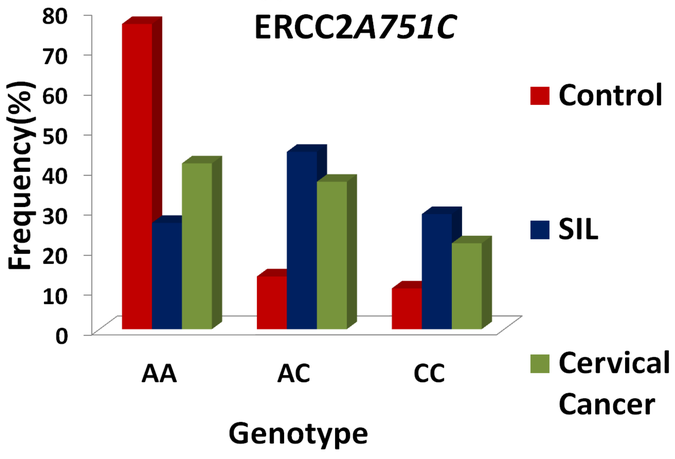
DNA was isolated from both blood and tissue samples using qiagen kit

ERCC2 was amplified by PCR using gene specific primers

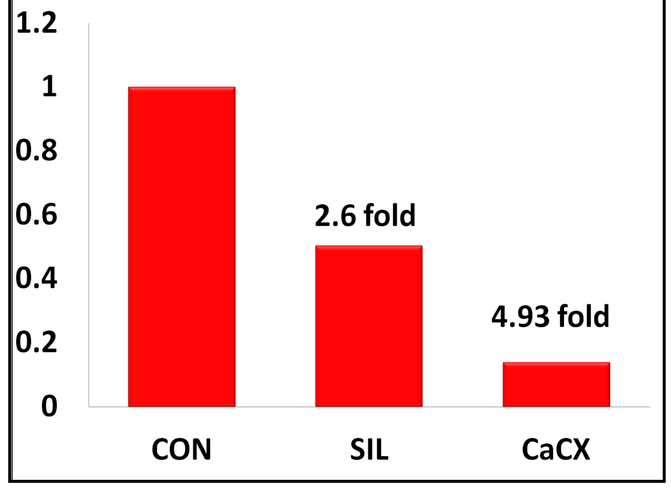
Amplified products was then subjected to RFLP by MboII

Results

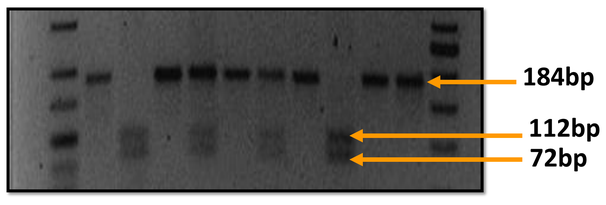
Genotype distribution of ERCC2*A751C*



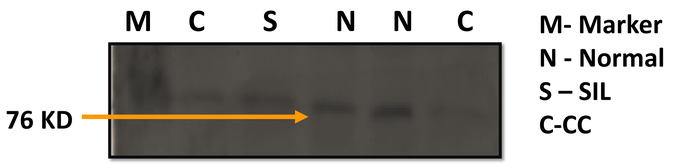
Fold Decrease In ERCC2 Transcript



ERCC2 751 Codon digestion with MboII (exon 23, Lys to Gln)

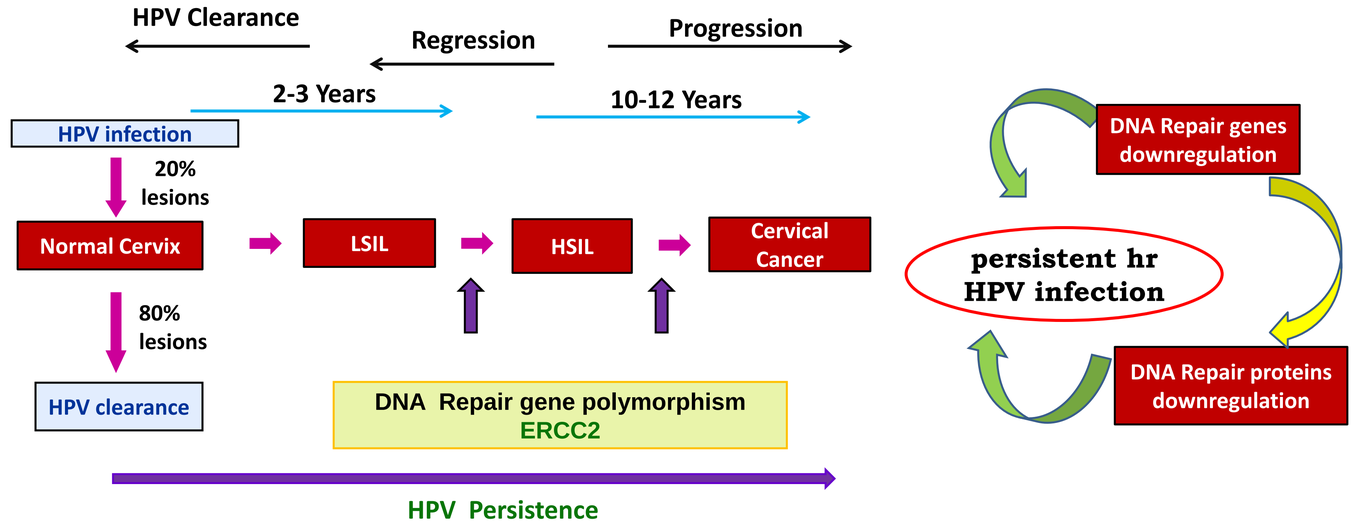


Western blot analysis of ERCC2 protein



Discussion & Conclusion

- ERCC2 homozygous variant genotype (CC) was significantly associated with CC risk (p=0.001 OR=5.9, 95%CI=1.6-21.8).
- The heterozygous (AC) and the combined heterozygous and variant genotype (AC+CC) also presented a significantly elevated risk of CC (p>0.05)..
- In SILs, the homozygous variant genotype (CC) was significantly associated with SIL risk (p=8.8. OR=8.9, 95%CI=2.3-33.1).
- A 2.63 and 4.93 fold decrease was observed in ERCC2 in SIL and cervical cancer (p=0.04, 0.008) as compared to control subjects.
- 61/65 (93.8%) of cervical tumor samples were positive for HPV. Out of these HPV positive samples 53/61 (86.8%) samples were infected with HPV16 type and 4/61 (6.5%) were infected with HPV18 type
- Thirty nine of the 45 SIL samples (86.6%) were positive for HPV 30/39 (76.9%) of SILs were infected with HPV16 type.
- Only 12/68 (17.6%) of the controls were positive for HPV.



The present study suggested ERCC2 as a predisposing factor in cervical precancer and increased risk of invasive cervical cancer. This implies that polymorphism of ERCC2 gene is associated with an early event in the progression to cervical cancer.