中文摘要

SOX1 基因甲基化对不同类型宫颈癌诊断价值的 meta 分析

研究背景：

目前，DNA 甲基化在宫颈癌诊断中的作用受到广泛关注，越来越多的基因被认为是宫颈癌的甲基化生物标志物。其中 SOX1 基因甲基化对宫颈鳞癌及其癌前病变的研究较多，然而宫颈腺癌的诊断一直是宫颈癌早期诊断的难题，SOX1 基因甲基化对宫颈腺癌是否也有类似的诊断效果，尚未有大样本量的统计分析证实。SOX1 基因甲基化对包括宫颈鳞癌和宫颈腺癌在内的不同类型宫颈癌的总体诊断效果尚需大量临床研究证实及系统评价。

目的：

通过 meta 分析的方法系统性评价 SOX1 基因甲基化对诊断不同类型宫颈癌的临床准确性，比较其对宫颈鳞癌和宫颈腺癌的诊断效果，并进一步用生物信息学分析 SOX1 基因甲基化与宫颈癌不同临床病理特征的相关性，为 SOX1 基因甲基化用于宫颈鳞癌和宫颈腺癌等不同类型宫颈癌的临床诊断提供理论基础。

方法：

立足于解决临床实际问题制定合适的检索策略以检索相关研究，用计算机全面检索 PubMed、Web of science、Embase、Cochrane Library、Clinical-Trail 等英文数据库，以及中国知网（CNKI）、维普中文科技期刊数据库（VIP）、中国生物医学文献数据库（CBM）和万方数据库等中文数据库，检索时间从数据库建立到 2022 年 12 月。由两位研究员分别按照纳入标准与排除标准对文献进行严格的独立筛选、质量评价与数据提取。通过合并敏感度(Sensitivity，Sen)、特异度(Specificity，Spe)、阳性似然比(Positive likelihood ratio，PLR)、阴性似然比(Negative likelihood ratio，NLR)、诊断比值比(Diagnostic odds ratio，DOR)、综合受试者工作特征曲线(Summary receiver operating characteristic curve，SROC)曲线下面积(Area under the curve，AUC)等准确性相关统计学指标来评估诊断价值，并进行 meta 回归分析、亚组分析及敏感性分析来探索异质性来源。本文利用 Review Manager 5.4 、Meta-Disc1.4 和 Stata14.0 软件进行 meta 分析。综合运用 DiseaseMeth、UALCAN、DNMIVD 和 MethSurv 在线数据库进行生物信息学分析，通过绘制甲基化差异箱式图和生存曲线来探究病理特征相关性和预后价值。

结果：

1.本文共纳入 13 篇文献中的 19 项研究，样本量共 3333 例，其中实验组 1054 例，对照组 2279 例。得出 SOX1 基因甲基化诊断不同类型宫颈癌的总体合并 Sen 为 0.87(95%CI：0.85-0.89)；合并 Spe 为 0.80(95%CI：0.78-0.82)；PLR 为 5.85(95%CI：3.84-8.90)；NLR 为 0.16(95%CI：0.13-0.19)；DOR 为 44.73(95%CI：25.38-78.82)；AUC 为 0.9322。

2.对其中 4 项宫颈腺癌的研究进行 meta 分析得出 SOX1 基因甲基化诊断宫颈腺癌的合并 Sen 为 0.88(95%CI：0.84-0.92)；合并 Spe 为 0.88(95%CI：0.81-0.92)；PLR 为 7.03(95%CI：1.54-32.00)；NLR 为 0.14(95%CI：0.09-0.23)；DOR 为 73.91(95%CI：5.13-1064.41)；AUC 为 0.9423。

3.对其中 7 项宫颈鳞癌的研究进行 meta 分析得出 SOX1 基因甲基化诊断宫颈鳞癌的合并 Sen 为 0.85(95%CI：0.80-0.89)；合并 Spe 为 0.96(95%CI：0.95-0.98)；PLR 为 18.88(95%CI：5.48-65.08)；NLR 为 0.15(95%CI：0.11-0.21)；DOR 为 132.41(95%CI：33.23-527.62)；AUC 为 0.9182。

4.通过 meta 分析对 SOX1 基因甲基化诊断不同类型宫颈癌的亚组分析中：样本量＜200 组对比样本量≥200 组亚组分析显示：（Sen=0.84）VS（Sen=0.91）；（Spe=0.87）VS（Spe=0.75）；(AUC=0.90）VS（AUC=0.97）。宫颈组织组对比宫颈细胞组亚组分析显示：（Sen=0.87） VS （Sen=0.86）；（Spe=0.64）VS（Spe=0.85）; (AUC=0.91）VS（AUC=0.94）。对照包含癌前病变组对比不含癌前病变组亚组分析显示：（Sen=0.86）VS（Sen=0.88）;（Spe=0.74）VS（Spe=0.96）; (AUC=0.91）VS（AUC=0.94）。

5.在不同数据库平台中进行生物信息学分析，SOX1 基因甲基化水平在宫颈癌样本中均明显高于正常样本。在不同宫颈癌病理类型的组间分析中，SOX1 基因甲基化水平：正常样本 VS 宫颈腺鳞癌（*P* =8.1670E-04）；正常样本 VS 宫颈鳞癌（*P* =2.7616E-09）；正常样本 VS 宫颈普通型腺癌（*P* =2.0099E-11）；正常样本 VS 宫颈黏液性腺癌（*P* =4.6768E-09）；正常样本 VS 宫颈内膜样腺癌（*P* =1.4954E-01）；宫颈普通型腺癌 VS 宫颈鳞癌（*P* =4.1035E-07）；宫颈普通型腺癌 VS 宫颈粘液性腺癌（*P* =2.7628E-03），且宫颈普通型腺癌中的甲基化水平最高。SOX1 基因甲基化水平在不同年龄、人种、肿瘤分级、肿瘤分期和有无肿瘤转移组间差异无统计学意义。

6.通过生物信息学分析发掘 SOX1 基因中 cg11750165 位点的甲基化水平对宫颈癌的总生存期有显著影响（P=0.0086<0.05），且该位点的高甲基化水平与宫颈癌更好的预后相关。

结论：

1.SOX1 基因甲基化对包括宫颈鳞癌和宫颈腺癌在内的不同类型宫颈癌均有较高的诊断价值。

2.生物信息学分析显示 SOX1 基因甲基化水平在宫颈鳞癌、腺鳞癌和部分 HPV 相关型腺癌中明显高于正常样本，且宫颈普通型腺癌中的甲基化水平最高。在 meta 分析中其对宫颈腺癌诊断的敏感度高于鳞癌，但特异性低于鳞癌，SOX1 基因甲基化的应用有望提高临床宫颈腺癌检出率。

3.SOX1 基因甲基化在大样本量的检测中敏感度更好，可以用于筛查人群中宫颈癌。

4.在样本类型上，宫颈脱落细胞相比宫颈组织在 SOX1 基因甲基化诊断宫颈癌中的特异度更高，为宫颈筛查中的分流管理提供了思路。

5.SOX1 基因甲基化在区分宫颈癌和正常人中的诊断价值高于区分宫颈癌与其癌前病变。

6.SOX1 基因甲基化程度与宫颈癌预后相关。

关键词：

SOX1 甲基化；宫颈鳞癌；宫颈腺癌；诊断；meta 分析；生物信息学

Abstract

Value of SOX1 Gene Methylation in Diagnosis of Different Types of Cervical Cancer ： a Meta-Analysis

Research Background:

At present,the role of DNA methylation in the diagnosis of cervical cancer has received extensive attention, and more and more genes are considered as methylation biomarkers of cervical cancer. There are many studies about SOX1 gene methylation on cervical squamous cell carcinoma and its precancerous lesions. However,the diagnosis of cervical adenocarcinoma has always been a difficult problem in the early diagnosis of cervical cancer. Whether SOX1 gene methylation has similar diagnostic effect on cervical adenocarcinoma has not been confirmed by statistical analysis with large sample size. The overall diagnostic effect of SOX1 methylation on different types of cervical cancer, including cervical squamous cell carcinoma and cervical adenocarcinoma, still needs to be confirmed by a large number of clinical studies and systematic evaluation.

Objective:

To systematically evaluate the clinical accuracy of SOX1 gene methylation in the diagnosis of different types of cervical cancer by using meta-analysis, compare its diagnostic effect on cervical squamous cell carcinoma and cervical adenocarcinoma. The correlation between SOX1 gene methylation and different clinicopathological characteristics of cervical cancer was further analyzed by bioinformatics. To provide theoretical basis for the clinical diagnosis of different types of cervical cancer such as cervical squamous cell carcinoma and cervical adenocarcinoma by SOX1 methylation.

Methods:

Based on solving practical clinical problems to formulate appropriate retrieval strategies to retrieve relevant studies, we used computers to comprehensively search English databases such as PubMed, Web of science, Embase, Cochrane Library, Clinical-Trail, and Chinese databases such as CNKI, VIP, CBM and Wanfang Database. The databases were searched from database creation to December 2022. Two researchers conducted strict independent screening, quality evaluation and data extraction according to inclusion criteria and exclusion criteria respectively. The data were extracted by combining Sensitivity, Specificity, PLR, NLR, DOR, SROC and

other accuracy-related statistical indicators to assess the diagnostic .The paper also uses meta-regression analysis, subgroup analysis and sensitivity analysis to explore sources of heterogeneity. The meta-analysis was conducted using a combination of Review Manager 5.4, Stata 14.0 software and Meta-Disc 1.4. Bioinformatics analysis was performed by applying DiseaseMeth, UALCAN, DNMIVD and MethSurv online databases, and the correlation of pathological features and prognostic value were explored by drawing methylation difference box diagram and survival curve.

Results:

1.A total of 19 studies from 13 publications with a total sample size of 3333 cases, including 1054 cases in the experimental group and 2279 cases in the control group, were included in this paper. The results showed that the combined Sen of SOX1 methylation in the diagnosis of cervical cancer was 0.87(95%CI: 0.85-0.89). Combined Spe was 0.80(95%CI: 0.78-0.82). PLR was 5.85(95%CI: 3.84-8.90);NLR was 0.16(95%CI: 0.13-0.19); DOR was 44.73(95%CI: 25.38-78.82); The AUC was 0.9322.

2.Analysis of four studies on cervical adenocarcinoma showed that the combined Sen of SOX1 methylation in diagnosis of cervical adenocarcinoma was 0.88(95%CI: 0.84-0.92). Combined Spe was 0.88(95%CI: 0.81-0.92). PLR was 7.03(95%CI: 1.54-32.00);NLR was 0.14(95%CI: 0.09-0.23); DOR was 73.91(95%CI: 5.13-1064.41); The AUC was 0.9423.

3.Analysis of 7 studies on cervical squamous cell carcinoma showed that the combined Sen of SOX1 methylation in diagnosis of cervical squamous cell carcinoma was 0.85(95%CI: 0.80-0.89). The combined Spe was 0.96(95%CI: 0.95-0.98). PLR was 18.88(95%CI: 5.48-65.08); NLR was 0.15(95%CI: 0.11-0.21). DOR was 132.41(95%CI: 33.23-527.62); The AUC was 0.9182.

4.In the subgroup analysis of SOX1 methylation in the diagnosis of different types of cervical cancer: sample size ＜200 versus sample size ≥200 showed: (Sen=0.84) VS (Sen=0.91); (Spe=0.87) VS (Spe=0.75); (AUC=0.90) VS (AUC= 0.97).Cervical tissue group versus cervical cell group showed: (Sen=0.87) VS (Sen=0.86); (Spe=0.64) VS (Spe=0.85); (AUC=0.91) VS (AUC= 0.94).Control group with precancerous lesion vs group without precancerous lesion showed: (Sen=0.86) VS (Sen=0.88); (Spe=0.74) VS (Spe=0.96); (AUC=0.91) VS (AUC= 0.94).

5.Through bioinformatics analysis in different database platforms, the methylation level of SOX1 gene in cervical cancer samples was significantly higher than that in normal samples. In the inter-group analysis of different pathological types of cervical cancer, the methylation level of SOX1 gene was: Normal VS Adenosquamous(*P*=8.1670E-04);Normal VS Squamous(*P*=2.7616E-09); Normal VS Endocervical(*P*=2.0099E-11);Normal VS Mucinous(*P*=4.6768E-09); Normal VS Endometrioid(*P*=1.4954E-01);Squamous VS Endocervical(*P*=4.1035E-07); Endocervical VS Mucinous(*P*=2.7628E-03), and the methylation level was the highest in adenocarcinoma of the usual type. There was no significant difference in the methylation level of SOX1 gene among different age, race, tumor grade, tumor stage and tumor metastasis.

6. Through bioinformatics analysis, it was found that the methylation level of cg11750165 locus in SOX1 gene had a significant impact on the overall survival of cervical cancer (*P*=0.0086<0.05), and high methylation level at this locus was associated with better prognosis of cervical cancer.

Conclusions:

1.SOX1 gene methylation has high diagnostic value for different types of cervical cancer, including cervical squamous cell carcinoma and cervical adenocarcinoma.

2. Bioinformatics analysis showed that the methylation level of SOX1 gene was significantly higher in cervical squamous cell carcinoma, adenosquamous cell carcinoma and some HPV-associated adenocarcinomas than in normal samples, and the methylation level was the highest in adenocarcinoma of the usual type. In the meta-analysis, its sensitivity to the diagnosis of cervical adenocarcinoma was higher than that of squamous cell carcinoma, but its specificity was lower than that of squamous cell carcinoma. The application of SOX1 gene methylation is expected to improve the clinical detection rate of cervical adenocarcinoma.

3.SOX1 gene methylation is more sensitive in the detection of large sample size, and can be used to screen cervical cancer in the population.

4.In terms of sample types, the specificity of exuded cervical cells in the diagnosis of cervical cancer by SOX1 gene methylation is higher than that of cervical tissues, which provides ideas for shunting management in cervical screening.

5.The diagnostic value of SOX1 methylation in distinguishing cervical cancer from normal subjects is higher than that in distinguishing cervical cancer from precancerous lesions.

6. The degree of methylation of SOX1 gene is related to the prognosis of cervical cancer.

Key Words:

SOX1 Methylation; Cervical Squamous Cell Carcinoma; Cervical Adenocarcinoma; Diagnosis; Meta-Analysis; Bioinformatics analysis

目 录

中英文缩略词

英文缩写	英文全称	中文全称
CC	Cervical Cancer	宫颈癌
SOX1	Sex Determining Region Y-box Protein 1	人性别决定区 Y 框-1
HPV	Human Papillomavirus	人类乳头瘤病毒
HR-HPV	High-risk Human Papillomavirus	高危型人类乳头瘤病毒
TCT	Liquid-based Cytology Test	液基薄层细胞学检查法
CpG	Cytosine-phosphate-guanine Dinucleotide	胞嘧啶磷酸鸟嘌呤二核苷酸
CIN	Cervical Intraepithelial Neoplasia	宫颈上皮内瘤变
TP	True Positive	真阳性
FP	False Positive	假阳性
FN	False Negative	假阴性
TN	True Negative	真阴性
CI	Confidence Interval	置信区间

第 1 章　引言

1.1　宫颈癌诊断方法的研究现状

宫颈癌是女性第四大常见癌症，也是全球女性癌症第四大常见死亡原因。全球每年诊断出 604,127 例宫颈癌新病例，341,831 人死于该疾病[1]。HPV 感染是宫颈癌最重要的相关因素，迄今已知有 150 多种病毒株。大多数宫颈癌病例与高危 HPV 感染有关，主要是 16 和 18 型。由于宫颈癌的病因较明确，通过定期筛查和疫苗接种，宫颈癌是高度可预防的。宫颈癌如果能及早发现，可以获得很好的生存预后和较高的生活质量[2]。

1.1.1　宫颈癌的筛查诊断方法

目前宫颈癌的筛查主要有巴氏涂片（CPS）、液基细胞学检查（LBC）、液基薄层细胞（TCT）检测、细胞学双重染色(p16/Ki67)、HPV DNA 检查和联合检查[2]。基于巴氏涂片的宫颈细胞学检测已经存在了半个多世纪，它的新形式是 LBC[3]。巴氏涂片试验的敏感度估计为 30-87%，特异度为 86%，假阴性结果的比例为25%-50%[4]。而 LBC 诊断宫颈癌的敏感度为61-66%，特异度为82-90%[5]。由此可见，CPS 诊断的敏感度较低，假阴性率较高，LBC 相对 CPS 的诊断敏感度较高。CPS 和 LBC 的检测准确性取决于当地医疗保健水平及病理学家的经验，因此因地区而异。液基薄层细胞（TCT）检测宫颈细胞的方法已经被广泛运用，TCT 对 CINs 有改善诊断效能的作用，尤其是对数目较少和体积较小的高级别鳞状上皮病变阳性诊断率有所提高。然而，一些细胞学阴性而 HPV 阳性妇女也有患 CIN 病变甚至宫颈癌的风险，因此 TCT 作为单独检测手段具有局限性[6]。细胞学双重染色(p16/Ki67)增加了检测高级别癌前病变的敏感性，是转化型 HPV 感染的标志，研究表明它的敏感性明显高于普通细胞学检测，但特异性较低[7]。已知宫颈癌发病与 HPV 感染关系密切，HPV DNA 检测可通过是否存在 HPV 感染来甄别宫颈癌的发病风险。HPV DNA 检测比宫颈细胞学更敏感，敏感度为 98.7%，特异度为 96%[8]。然而有些一过性 HPV 感染并不会导致宫颈癌，因此 HPV 检测的高敏感性可能导致过多假阳性结果产生，一些患者可能需要进一步检查，