**Screening of diagnostic markers:**

1. Next-generation sequencing (Early- and mid-stage ESCC and paired adjacent normal tissues).
2. Bioinformatics analysis (volcano map; miRDB; TargetScan; miRanda).
3. Detection of clinical tumor markers (SCC; CEA; CA19-9).

**Exclusions** (n=17):

(1) Combined with liver, kidney and other organ diseases (n=2);

(2) The tumor stage is stage IV (n=7);

(3) Tumor history (n=5);

(4) Those who have not signed the informed consent (n=3).

**Verification cohort**

Taizhou People's Hospital

(Early and mid-stage ESCC= 45, Health control =45)

**Testing cohort**

Huaian Hospital of Huaian City

(Early and mid-stage ESCC= 75, Health control =75)

**Establishment of early- and mid-stage ESCC diagnostic regression model** (including miRNA-15b-5p, miRNA-424-5p, miRNA-497-5p, miRNA-363-5p, miRNA-195-5p, and SCC).

**Included subjects**

(n=257)

**Subjects with reference test** (Early and mid-stage ESCC=120, Health control =120)

Validation group (Prognosis model) - The Affiliated Kunshan Hospital of Jiangsu University (ischemic stroke=149) and The Second Affiliated Hospital of Soochow University (ischemic stroke=133)