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Variation of autosomes and X chromosome STR in breast cancer and gynecological cancer tissues

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Abstract: This study analyses 1000 cases of patients with breast cancer and 2000 cases of patients with gynecological cancer (1000 cases of malignant tumor, 1000 cases of benign tumors), where breast cancer and malignant tumor patients comprise the observation group, while patients with benign tumors comprise the control group. Through DNA extraction, STR genotyping and variation verification, microdissection, individual STR mutation rate and loci STR mutation rate of the two groups of patients were calculated. Results show that there are no significant (P > 0.05) differences in the STR variation of autosomes and X chromosome between patients in the observation group and those in the reference group. However, significant (P < 0.05) intergroup differences were found for STR variation typing between patients with malignant and benign tumors. Using STR genotyping for autosomes and X chromosomes, gynecological cancer patients were found to be more likely to mutate, with a clear relationship between STR variation and tumor differentiation degrees. The study on the variation analysis of autosomes and X chromosome STR in breast and gynecological cancer tissues is expected to have a high application value when applied to medical research and identification processes.

Keywords: gynecological cancer, breast cancer, autosomes, X chromosome, STR variation typing

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2 Data and Methods

2.1 General data

A total of 3000 cases of female cancer patients were selected as research subjects between January 2012 and January 2015, including 1,000 cases of breast cancer, 1,000 cases of malignant tumors, and 1000 cases of benign tumors. All subjects had a clear understanding of purpose and significance of this study, and voluntarily provided tumor samples. After collection, samples were kept frozen at -70°C. Among the 1000 cases of patients with malignant tumors, there were 321 cases of ovarian cancer, 214 cases of cervical cancer, 256 cases of vulvar cancer, and 209 cases of endometrial cancer. Sample data were organized according to patients' age, tumor type, clinical stage, and tumor differentiation.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee.

2.1.1 DNA extraction

DNA was extracted from gynecological and breast cancer tissues, as well as from adjacent non-tumorous tissue using an animal tissue / cell genomic DNA extraction kit (Solarbio, China) and a paraffin-embedded tissue DNA extraction kit (Qiagen, Germany). Genomic DNA from blood was extracted using a blood genome extraction mini kit (Qiagen, Germany). DNA was quantified with a ND-1000 protein nucleic acid quantometer (Quawell, USA).

2.1.2 STR typing

A PowerPlex21 system and Argus X12 kit were adopted for DNA amplification. Electrophoresis typing of PCR products was conducted with ABI3130 Genetic Analyzer, Using GeneMapperTM v3.2, collected data were analyzed, and STR typing of malignant tumors, benign tumors, and blood groups were determined. Normal tissue and DNA typing of the same individual, DNA typing of tumors and normal tissues of the same individual, STR variation sites, and fractal categories were observed and recorded [4].

2.1.3 STR variation verification

The experiments on all 3000 samples were repeated twice. STR validation variation was undertaken with mutual authentication between kits and single-locus amplification.

2.1.4 Microdissection

Microdissection on STR variation position was performed, and DNA of tumor cells and normal mesenchyme was extracted [5].

2.2 Statistical methods

Data processing and analysis was performed using SPSS19.0 statistical software, with results reported as mean ± average (\( \bar{x} \pm s \)). Statistically significant differences were determined at P < 0.05 using Student’s t-test.

3 Results

The rate of 4 mutation types of autosomal and X chromosomal STR in gynecologic cancer is shown in Table 1. No variation was observed in STR typing of PB (peripheral blood) samples of malignant and benign tumors, although STR mutation rates differed between in GCST (gynecological cancer STR tissue) samples. Individual detection rates were recorded for three STR variation types with genotype changes, (P > 0.05, without statistical significance).

The locus detection rates in autosomal STR (P < 0.05 with statistical significance) are shown in Table 2. The research results show that for both autosomes and X chromosomes, there were no significant differences in STR variation of gynecological tumor tissue of different tumor types, or between tissue types and differentiation degrees. In gynecological tumor tissues, six samples exhibited autosomal SRT variation with more than 3 loci, while five samples exhibited X chromosomal SRT variation with more than 3 loci. Of the 33 total loci, there were 9 samples with SRT variation with more than 3 loci, and a single sample with 13 loci in simultaneous STR variation.

The DNA typing results show that no STR variations of loci were observed for autosome and X chromosome of benign gynecologic tumor tissue. The gene typing result is consistent with that of control group. The histology of breast cancer samples is shown in Figure 1.
Four STR variation types have been identified in gynecological tumor tissue, including Aadd, Anew, LOH and pLOH [5-6]. However, Aadd, Anew, LOH are the most likely to cause gene mutations (STRGA) in clinical settings, which may lead to flawed judgments by researchers. The research results show that mutation rates of STR loci of gynecological tumors is in the range of 0 to 9.67%. Previous clinical research has shown [6] that loci such as Penta E, D5S818, FGA have relatively higher autosome STR mutation detection rates, which were 6.44%, 9.66%, 8.04%, respectively. DXS10146 in X-STR had the strongest mutation rate which reached 9.68%. There were no STR mutations shown in the loci of AMEL, Penta D and HPRTB in this research. The STR variation of other loci is significantly higher than the average mutation rate of DNA, which may result in variations in allele typing. However, no mutation in the STR loci of benign tumor tissues have been observed. A comparison between autosome STR variation in relatively benign and malignant gynecological tumors does not reveal significant differences. In X-STR, STR variation between the two is not significantly different (P > 0.05). A comprehensive comparison of STR variation in autosomes and X chromosome reveals that there is a

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>Autosomal STR mutation rate (%)</th>
<th>95%CI</th>
<th>X chromosomal STR mutation Rate (%)</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper limit</td>
<td>Lower limit</td>
<td>Upper limit</td>
<td>Lower limit</td>
</tr>
<tr>
<td>p LOH</td>
<td>3.31</td>
<td>4.76</td>
<td>1.86</td>
<td>7.62</td>
</tr>
<tr>
<td>Aadd</td>
<td>1.00</td>
<td>1.87</td>
<td>0.11</td>
<td>2.15</td>
</tr>
<tr>
<td>Anew</td>
<td>0.85</td>
<td>1.44</td>
<td>0.23</td>
<td>1.77</td>
</tr>
<tr>
<td>LOH</td>
<td>2.86</td>
<td>4.62</td>
<td>1.06</td>
<td>0.95</td>
</tr>
<tr>
<td>STR_{Xa}</td>
<td>4.68</td>
<td>6.89</td>
<td>2.49</td>
<td>4.85</td>
</tr>
</tbody>
</table>

Table 2 The STR mutation rate of different tumor types in gynecological cancer.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Number</th>
<th>Number</th>
<th>STR mutation rate (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Autosome</td>
<td>X chromosome</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>206</td>
<td>31.59</td>
<td>31.59</td>
<td>48.36</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>185</td>
<td>31.26</td>
<td>12.43</td>
<td>43.76</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>120</td>
<td>142</td>
<td>130</td>
<td>165</td>
</tr>
<tr>
<td>Vulvar cancer</td>
<td>6</td>
<td>150</td>
<td>0</td>
<td>150</td>
</tr>
</tbody>
</table>

Fig. 1. Histology of breast cancer samples. A) 2 months carcinogenesis, B) 6 months carcinogenesis, C) 10 months carcinogenesis, D) 12 months carcinogenesis.
significant difference (P <0.01) in STR variation between benign and malignant gynecological tumor tissues.

Among the 1000 cases of breast cancer patients in this survey, the average STR loci mutation rate was in the range of 0 to 4.76%, suggesting that loci mutation frequency for STR to variate is relatively low. Autosomes are likely to have fewer loci exhibiting STR variation than that of X chromosomes [7-8]. Additionally, autosomal STR variation is negligible throughout breast cancer differentiation stages. Therefore, staging detection is not likely to play a significant role in clinical settings [9]. STR exist in all regions of the human genome including coding, control, and intron regions. Accordingly, STR variation can change the transcription and translation of rates of a given gene. This STR polymorphism found in breast cancer-related genes (androgen receptor gene, estrogen receptor gene, transcription factor E2F-4 gene, cytochrome C P450 gene, insulin-like growth factor I gene, breast cancer amplify gene 1, interferon-γ gene) will affect gene translation, and may even be a cause of breast cancer.

The results of this study fully demonstrate that loci level of STR mutation in tumor tissues of patients with gynecological tumor is significantly higher than that in tumors of breast cancer patients, loci number for gynecological tumors to have STR variation is significantly higher than that of breast cancer, loci mutation rate of gynecological tumor is relatively higher than that of the same loci of breast cancer [10]. In gynecological tumors, variation usually occurs in autosomes STR loci, while in breast cancer, X-STR variation is more common.

In summary, the results of this study show that patients with gynecological tumors are more likely to have variations in STR whose variation degree is closely associated with the extent of tumor differentiation and staging. Therefore, analyzing the STR variations of autosome and X chromosome in breast cancer and gynecological tumor tissues is of vital significance to making definite diagnosis of breast and gynecological cancers.

Conflict of interest: Authors state no conflict of interest.

References