GENETIC FEATURES OF NO Generating SYSTEMS AND RESISTANT TO EHRLICH ASCITES CARCINOMA

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In the immune system:

- TUMOR
- MACROPHAGE
- An individual genotype

Background:

1. Vulnerability to tumor development
2. Genetic predisposition
3. NO and effectiveness of the NO-generating system
4. Antitumor immune defense
HYPOTHESES:
the vulnerability to tumors could be
predetermined by genetic characteristics
of the macrophage NO-generating
system

DO NOT DOUBT!!!

I DOUBT VERY MUCH!
THREE MAIN OBJECTIVES were designed to achieve the study goal:

1) to evaluate susceptibility of different mouse substrains, C57BL/6\textit{J} and C57BL/6\textit{N}, to development of Ehrlich ascites carcinoma (EAC);

2) to study the role of NO in susceptibility to development of tumor using a NO scavenger, an inducible NO synthase inhibitor, and a NO donor;

3) to identify the macrophage phenotype and to evaluate the effectiveness of NO generation in macrophages from mice of different substrains.
OUR FIRST OBJECTIVE WAS TO EVALUATE SUSCEPTIBILITY OF DIFFERENT MOUSE SUBSTRAINS TO DEVELOPMENT OF EHRLICH ASCITES CARCINOMA (EAC)
C57BL/6N mice were significantly more resistant to the development of tumor than C57BL/6J mice.

The resistance of mice to EAC was assessed by survival time. An i.p. injection of EAC cells was given, and the survival time after the injection was measured. The graph shows the cumulative proportion surviving for both C57BL/6N and C57BL/6J mice. C57BL/6N mice showed a longer survival time, with ~19 days compared to ~15 days for C57BL/6J mice.
OUR SECOND OBJECTIVE WAS TO STUDY THE ROLE OF NO IN SUSCEPTIBILITY TO DEVELOPMENT OF TUMOR USING A NO SCAVENGER, AN iNOS INHIBITOR, AND A NO DONOR
INCREASED NO PRODUCTION PROLONGS WHILE REDUCED NO PRODUCTION SHORTENS SURVIVAL OF MICE WITH TUMOR

C57BL/6N mice with tumor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lifespan, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>+ NO, 10%</td>
</tr>
<tr>
<td>ITU</td>
<td>- NO, - 23%</td>
</tr>
<tr>
<td>PTIO</td>
<td>- NO, - 23%</td>
</tr>
<tr>
<td>control</td>
<td></td>
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</tbody>
</table>

C57BL/6J mice with tumor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lifespan, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>+ NO, +26%</td>
</tr>
<tr>
<td>ITU</td>
<td>- NO, - 17%</td>
</tr>
<tr>
<td>PTIO</td>
<td>- NO, - 17%</td>
</tr>
<tr>
<td>control</td>
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</table>
OUR THIRD OBJECTIVE WAS TO IDENTIFY THE MACROPHAGE PHENOTYPE AND TO EVALUATE THE EFFECTIVENESS OF NO GENERATION IN MACROPHAGES FROM MICE OF DIFFERENT SUBSTRAINS
NO PRODUCTION WAS HIGHER IN MACROPHAGES OF C57BL/6N MICE THAN IN MACROPHAGES OF C57BL/6J MICE

<table>
<thead>
<tr>
<th>Substrain</th>
<th>Survival after tumor induction, days</th>
<th>Indices of NO generation systems</th>
<th>M1 marker, CD 80</th>
<th>M2 marker, CD206</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR C57BL/6N</td>
<td>19.5±0.5</td>
<td>Nitrite, µmol</td>
<td>80.6±5.6%</td>
<td>52.7±4.9%</td>
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<td></td>
<td></td>
<td>Basal conditions</td>
<td>Stimulated conditions</td>
<td></td>
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<tr>
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<td></td>
<td>45.7±1.34</td>
<td>74.4±1.26</td>
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<td></td>
<td>iNOS</td>
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<tr>
<td></td>
<td></td>
<td>Basal conditions</td>
<td>Stimulated conditions</td>
<td></td>
</tr>
<tr>
<td>LR C57BL/6J</td>
<td>15.6±0.6*</td>
<td>Nitrites, µmol</td>
<td>4.0±0.3%*</td>
<td>58.8±4.7%</td>
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<td>Basal conditions</td>
<td>Stimulated conditions</td>
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<tr>
<td></td>
<td></td>
<td>29.6±1.43*</td>
<td>52.2±1.28*</td>
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<td>iNOS</td>
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The macrophage phenotype of C57BL/6N mice can be defined as a more distinct proinflammatory M1 phenotype compared with macrophages of C57BL/6J mice.
NITRIC OXIDE PLAYS A DUAL ROLE IN CARCINOGENESIS

**NO can exerts anti-tumor effects**

- by inhibiting synthesis of anti-apoptotic Bcl-2
- by increasing expression of proapoptotic Bax
- by increasing expression of proapoptotic p53

**NO can exerts pro-tumor effects**

- by nitrosylating caspases
- by activating HSP70 synthesis
- by inhibiting of Complex IV in mitochondria

**NO**

**TUMOR**
NO EXERTED AN ANTI-TUMOR EFFECT

possess a more expressed PI-M1 phenotype
What determines the genetically higher potency of NO-generating systems in macs of C57BL/6N mice compared with macs of C57BL/6J mice?

The fact that NO plays an important role in the prolonged survival under EAC, makes it promising to develop new approaches to anti-tumor treatment by manipulating NO in the tumor.
1. C57BL/6\textit{N} mice were significantly more resistant to the development of EAC than C57BL/6\textit{J} mice as shown by the parameters of survival duration.

2. Increased NO production prolongs while reduced NO production shortens survival of mice with EAC.

3. Macs of HR to tumor C57BL/6\textit{N} mice have more powerful NO generating system and a more distinct M1 phenotype than macs of LR C57BL/6\textit{J} mice.
HYPOTHESES:
the vulnerability to tumors is predetermined by genetic characteristics of the macrophage NO-generating system

I HAVE NO DOUBT!!!

RESEARCHERS

DO NOT DOUBT!!!
I appreciate deeply:

1. my colleagues from my Lab, doctors Lyamina and Kalish and students from department of Pathophysiology for cooperation in this research.

2. the Ministry of Science and Education of Russia for financial support. Agreement №14.604.21.0020, Unique identifier applied research RFMEFI60414X0020
Thank you for your attention