Toxicity analysis of PbS QDs using nano-sized vesicles (exosome) secreted from HEK293 cells

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**Quantum dots**

- **Nanocrystals**
- 2-10 nm diameter, small enough to exhibit quantum mechanical properties
- **Fluorophore**
- Easily modified by polymers or chemicals with such as –SH
- **Semiconductors, agents for medical imaging**

Exosomes

Roles of exosome contents
- Cell-to-cell signaling
- Membrane trafficking
- Representing the disease status and pathological condition of cellular origin
Properties of exosomes in biological signaling

- Released from most types of mammalian cells
- Different cells release different exosomes.
- Potential source of biomarkers for tissue conditions
- The encapsulated molecules by exosomes can be delivered over long distance.
- They play a key role in cell-cell communication during disease progress such as cancer and neurodegeneration.
- Isolated from circulating fluids: serum, urine, CSF and cell culture media
Toxicology with exosomes

* provides new circulating biomarkers for in vivo and in vitro
* makes possible to predict the cellular and molecular mechanism of toxicity efficiently
* is an emerging field for high-throughput screening of disease biomarkers
* is applied to human toxicology
Aims of this study are:

- To provide nanotoxicological data for PbS QDs
- To propose a toxicologic pathology using exosome biomarkers
1 Preparation of MPA-coated PbS

2 Exposure of PbS-MPA to HEK293 cells

3 Exosome isolation

4 Analysis of molecular markers: miRNA & proteins

5 Identification of Induced & depressed molecules

6 Functional analysis

7 Prediction of toxicologic pathology in vitro
PbS quantum dots with < 5 nm particle sizes were prepared by the colloidal chemistry method according to the theory of fast nucleation at high temperature and slow growth at low temperature. Sodium sulfide was used as a sulfur precursor (odorless and less noxious). Oleic acid was used as a stabilizing agent to control the particle growth and it assisted in the formation of mono-dispersed PbS QDs.
Surface modification PbS by MPA

3-mercaptopropionic acid (MPA)

TEM image

DLS analysis
FT-IR analysis

![Graphs showing FT-IR analysis of PbS and PbS-MPA](image)

PbS

PbS-MPA
Cytotoxicity of PbS-MPA

**Graphs:**
- **HEK293**
- **TCMK1**
- **THP1**
- **ALM12**

**Y-axis:** Cell proliferation (%)

**X-axis:** µg/ml

**Legend:**
- TCMK1
- THP1
- ALM12

**Data Points:**
- 0, 1.5, 15, 150 µg/ml

**Observations:**
- Cell proliferation decreases with increasing concentration of PbS-MPA.
Isolation and characterization of exosomes

- **Bio-TEM**
- **DLS**
- **Exosome markers**
  - CD63 (53KD)
  - CD9 (28KD)

System Bioscience Inc.
http://www.systembio.com/
In SBI miRNA analysis kit, 380 primers found in exosomes were provided.
Differentially expressed exosome miRNAs (DEGs)

Selection criteria:
1) Increment or decrement simultaneously in both of 5 and 50 µg/ml
2) Cut-off for 2-fold changes in 5 or 50 µg/ml
3) \( p < 0.05 \)

Log\(_2\)(\(\Delta Ct\))

5 µg/ml
50 µg/ml
### Top functional networks

<table>
<thead>
<tr>
<th>Diseases and disorders</th>
<th>p-value</th>
<th># Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>6.52E-04 - 4.38E-02</td>
<td>11</td>
</tr>
<tr>
<td>Organismal Injury and Abnormalities</td>
<td>6.52E-04 - 2.51E-02</td>
<td>8</td>
</tr>
<tr>
<td>Reproductive System Disease</td>
<td>2.85E-03 - 4.95E-02</td>
<td>4</td>
</tr>
<tr>
<td>Inflammatory Response</td>
<td>2.18E-03 - 2.79E-02</td>
<td>4</td>
</tr>
</tbody>
</table>

### Associated Network Functions

<table>
<thead>
<tr>
<th>Cancer, Organismal Injury and Abnormalities, Reproductive System Disease</th>
<th>Score</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

By Ingenuity Pathway Analysis (IPA)
2D gel electrophoresis of exosome proteins

1) 1<2<3 or 1>2>3  
2) 1 vs. 2 or 1 vs. 3, 2-fold cut off

1 Control

2 5 µg/ml PbS-MPA

3 50 µg/ml PbS-MPA
Spot selection (29 spots)

A: Increased changes (19)
B: Decreased changes (10)
Protein spots identified by Maldi-TOF and PMF analysis

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein</th>
<th>Expression</th>
<th>Function</th>
<th>Response in cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>9305</td>
<td>Leucine-rich repeat-containing protein 23 isoform b (IRR23b)</td>
<td>Increase</td>
<td>Exosome protein</td>
<td>Increase</td>
</tr>
<tr>
<td>5204</td>
<td>Keratin 5 (KRT5)</td>
<td>Decrease</td>
<td>Breast cancer biomarker (negative relationship)</td>
<td>Decrease</td>
</tr>
<tr>
<td>8603</td>
<td>Unnamed protein product</td>
<td>Decrease</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8703</td>
<td>Albumin-like</td>
<td>Decrease</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Confirmation of exosome biomarkers

In Exosomes

- LRR23b
- KRT 5
- p53
- CD9

Graphs showing LRR23b, KRT 5, and p53 levels in exosomes with QD-in concentrations of 0, 5, and 50 µg/ml.
Events in the origin of the exosomes, HEK293 cells: expression of protein biomarkers
Comet assay responses as indicators of carcinogen exposure.
Events in the origin of the exosomes, HEK293 cells: expression of cancer-related biomarkers (mRNA)

**Human IL-8**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Relative Ratio</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>QD-in 5 µg/ml</td>
<td><strong>3</strong></td>
</tr>
<tr>
<td>QD-in 50 µg/ml</td>
<td><strong>8</strong></td>
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</table>

**Human CXCL5**

<table>
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<th>Condition</th>
<th>Relative Ratio</th>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>QD-in 5 µg/ml</td>
<td><em>5</em></td>
</tr>
<tr>
<td>QD-in 50 µg/ml</td>
<td><strong>10</strong></td>
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**Human p53**

<table>
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<th>Condition</th>
<th>Relative Ratio</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>QD-in 5 µg/ml</td>
<td><em>2</em></td>
</tr>
<tr>
<td>QD-in 50 µg/ml</td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>

*P<0.01, **P<0.001 vs Control
Conclusion

* The exosome miRNA profiles for PbS-MPA to HEK293 cells showed 15 DEGs. These were primarily involved in cancer and organismal injury and abnormalities.

* LRR23b and KRT5 were identified as biomarkers of exosome proteins for PbS-MPA exposure. These proteins are also known as cancer biomarkers.

* Comet assay clearly showed that DNA fragmentation was occurred, and supported the carcinogenic activity of PbS-MPA QDs.

* The exosome derived biomarkers could represent the toxicological response of origin cells.

1. A toxicological response could be identified by genomic and proteomic analysis for secreted exosomes.

2. The exosome-based analysis could provide an effective tool for high-throughput screening (HTS) of biomarkers involved in possible toxicology.

3. The HTS of exosome biomarkers will be more efficient than that of the whole cells, because 1) they have less number of molecular contents, 2) are expected to secret critical biomarkers to represent the cellular state and communicate with other cells.
Thank you for your attention