Varying Opinions on the Authenticity of a Human Midgut Carcinoid Cell Line – Letter

Lee M. Ellis, Shaija Samuel, and Eric Sceusi

In 2007, our laboratory published an article in *Clinical Cancer Research* reporting on the development of a human midgut carcinoid tumor cell line (1). This cell line has now been shared with over 20 laboratories around the world. Investigators in the field of neuroendocrine tumor research are aware that there are very few human carcinoid cell lines available for use in vitro and for murine studies. At the time of publication of this article, we believed that we had developed the first human midgut carcinoid cell line in North America. These cells expressed dense neurosecretory granules on electron microscopy, VMAT-1/2, and somatostatin receptors (1). Recently, we performed short tandem repeat genotyping of the CNDT cell line, and we were unable to match this cell line to any other cell line in our own database at M.D. Anderson Cancer Center, The Johns Hopkins database, or the American Type Culture Collection database. This, in some ways, was reassuring as these databases contain data on thousands of cell lines, and we could rule out contamination of our CNDT cells by other well-characterized cell lines. However, we then tried to match our cell line with frozen carcinoid specimens in our tissue bank, and unfortunately, we were unable to match our short tandem repeat genotype to the carcinoid tumor that we thought was the source of this cell line. The short tandem repeat data for CNDT2 is listed below:

<table>
<thead>
<tr>
<th>CSF1PO</th>
<th>D13S317</th>
<th>D16S539</th>
<th>D18S51</th>
<th>D19S433</th>
<th>D21S11</th>
<th>D2S1338</th>
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<tr>
<td>8,11</td>
<td>8,11</td>
<td>12,13</td>
<td>11,13</td>
<td>14</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>D5S25</td>
<td>D5S818</td>
<td>D7S820</td>
<td>D8S1179</td>
<td>FGA</td>
<td>TH01</td>
<td>TPOX</td>
</tr>
<tr>
<td>16</td>
<td>11,14</td>
<td>8,10</td>
<td>9,13,14</td>
<td>6,10</td>
<td>10,11</td>
<td>25</td>
</tr>
</tbody>
</table>

Several investigators who have obtained this cell line have questioned the authenticity of its neuroendocrine background. At the Carcinoid and Neuroendocrine Tumor Scientific Forum 2010, a joint conference sponsored by the Verto Institute, The Raymond and Beverly Sackler Foundation, and The Caring for Carcinoid Foundation, held in Boston, MA, May 7, 2010, there were mixed opinions on the neuroendocrine tumor origin of this cell line, as summarized below. Some of these findings were in contrast with our own findings, whereas other observations were unique from those found in our studies.

- Problems with identifying chromogranin in the CNDT2 cells; however, investigators were able to identify somatostatin receptors associated with neuroendocrine tumor cells. Despite the lack of other markers, they thought the cell line was a neuroendocrine cell line.
- CNDT2 cells did not express ASCL1, which, the investigators stated, is expressed in all neuroendocrine tumor cell lines they had tested to date.
- No functional or structural features of neuroendocrine differentiation. Specifically, no secretory granules were identified by electron microscopy. Extensive panels of antibodies for neuron-specific enolase, chromogranin, synaptophysin, 5-HT, serotonin, CCK, GHRH, and a few others were negative by immunocytochemistry and were not present in supernatants.
- One group was able to increase the transduction of CNDT2.5 cells utilizing somatostatin receptors as a target, whereas transduction with nonspecific vectors was less. This suggests the presence of functional somatostatin receptors on the cell surface.
- One investigator hypothesized that the procedure used to generate the immortal carcinoid cell line may have imposed expedited "unnatural selection" for these indolent carcinoid cell lines. As a result, additional somatic mutations were generated and enhanced its survival to grow aggressively. This investigator noted that neuroendocrine-mesenchymal transition was similar to epithelial-mesenchymal transition, which is known to be a crucial process in both cancer and developmental biology. These cellular transitions tell us that cells are capable of being rewired fairly rapidly and assume very different cellular phenotypes to reflect the microenvironments that they are in. Sometimes, additional somatic mutation(s) may lead to irreversible neuroendocrine-mesenchymal transition.
- Gene expression profiling by one investigator failed to observe substantial expression of genes expected to be present in neuroendocrine cells. This may not be sufficient to conclude that the line is or is not of.

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carcinoid origin, but it did raise questions among these researchers about its reliability.

- In another group’s experiments, CNDT2.5 expressed hallmarks of what they considered carcinoid: by using high-performance liquid chromatography, they showed that the cells contained serotonin or serotonin metabolites at levels similar to BON cells. By end-point reverse transcription-PCR, the cells expressed a message for voltage-dependent Ca\textsuperscript{2+} channels; the \( \alpha_{1D} \) and \( \alpha_{1C} \) L-type transcripts, as well as 1E (R-type), were identified in CNDT2.5 lysates, but at much lower levels compared with BON. Also, in contrast with BON, the researchers were never able to show functional expression of these channels. Overall, the investigators believed that these cells had carcinoid features.

Researchers are advised to keep in mind the questions and skepticism of investigators in the field regarding the neuroendocrine authenticity of this cell line when reading articles that report on its use.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**Reference**

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