Use of Exfoliated Cells from Target Tissues to Predict Responses to Bioactive Food Components

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ABSTRACT A host of bioactive food components have been proposed to promote health and reduce the risk of disease states. It is clear that not all individuals respond identically to these essential and nonessential food components. Genetic polymorphisms may influence absorption, metabolism and accumulation of bioactive food components, thereby influencing their actions in target tissues. Unfortunately, serum concentrations of bioactive food components may not correlate with tissue concentrations and may therefore under- or overestimate the response in target tissues. Exfoliated cells may be useful to assess the actions of nutrients in specific tissues. Although not extensively examined, evidence already suggests the usefulness of these cells in predicting changes in gene expression, DNA methylation, DNA damage, protein expression and accumulation of dietary components. Although there are limitations on the collection of exfoliated cells, the inaccessibility of tissues they can represent raises intriguing possibilities for their ability to predict the outcome of nutritional intervention studies. J. Nutr. 133: 1769–1772, 2003.

KEY WORDS: • exfoliated cells • biomarkers • cancer • colon • lung • breast

Dietary habits are fundamental to achieving a person’s genetic potential and likely to reduce the risk of a myriad of disease conditions. A large number of bioactive food components may account for these observations. Although blood and its associated cells have frequently been used to evaluate the adequacy of the diet, their evaluation may not always predict responses in target tissues. However, it is often difficult if not impossible to obtain samples of the tissue of interest noninvasively. Thus, there is a need to validate the utilization of surrogate markers that would be indicative of the accumulation and activity of bioactive food components in cells in target tissues. Exfoliated or sloughed cells may be useful.

Exfoliated Cells as Surrogates for Cells in Target Tissues. Exfoliated cells hold strong potential as a tool for monitoring human exposure to bioactive food components in target tissues because they can be collected easily from human samples (Table 1). Examples include colonic epithelial cells obtained from stool samples or from gastric lavage, bladder urothelial cells present in urine samples, airway epithelial cells in sputum and buccal mucosal cells obtained by rinsing the mouth. In nonlactating women, epithelial cells have been obtained by ductal lavage, from nipple aspirate fluid and from fine needle aspiration of the breast parenchyma (1–4). Furthermore, the cellular profile of breast ductal fluid obtained by nipple aspiration in healthy premenopausal women was found not to vary during the menstrual cycle, indicating that future studies utilizing these samples may be performed independent of the phase of the cycle (5). Human breast milk has also been shown to be an excellent source of luminal epithelial cells from a cohort of lactating women (6). These cells reflect what actually occurs in the breast and are the cells from which most breast cancer arises. They are also more easily accessible, readily available and noninvasively collected compared with those from surgically excised tumor tissue and reduction mammaplasty. Thus, various samples may serve as sources of representative ductal epithelial cells for studies of exposure of the mammary gland, colon, lung and bladder to bioactive food components.

Although it has not been studied extensively, human semen is a potential source of prostate epithelial cells. It has been demonstrated that prostate cells in human semen can be characterized and used in the clinical diagnosis of prostate cancer (7). Diploid, cytokeratin 18-positive epithelial cells are found in all semen specimens regardless of disease status (7). More information is required about the yield and quality of prostate epithelial cells present in semen.

DNA, RNA and protein isolated from exfoliated cells have been analyzed for various types of genetic and epigenetic changes (Table 2). For example, mammary epithelial cells present in nipple aspirate fluid have been analyzed for DNA amplification of several microsatellite regions using polymerase chain reaction and for protein expression using two-dimensional gel electrophoresis (13), thus demonstrating that exfoliated cells may be useful for genetic analysis.

Accumulation and Metabolism of Bioactive Food Components. Exfoliated cells have been utilized for the measurement of the accumulation of bioactive food components. Exfoliated human colonic epithelial cells have been shown to accumulate tocopherols, retinol and carotenoids in response to dietary alterations (36). Turnlund and co-workers (37) observed that the mean copper content in colonic cells isolated from stool samples of healthy men increased significantly in response to copper supplementation, thus demonstrating that exfoliated cells can be utilized to measure the concentration of bioactive food components. However, because the concentration of these nutrients in colonic tissue was not measured, the utilization of the exfoliated cells as a surrogate for the concentration in colonic tissue remains unresolved in this case.

Although blood and blood constituents have frequently been used to evaluate the response to bioactive food components, the concentration of these agents in the blood and in

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Exfoliated epithelial cells</td>
</tr>
<tr>
<td>Lung</td>
<td>Exfoliated epithelial cells</td>
</tr>
<tr>
<td>Breast</td>
<td>Mammary gland ductal cells</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Genetic and Epigenetic Changes</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA amplification</td>
<td>Mammary epithelial cells</td>
</tr>
<tr>
<td>Protein expression</td>
<td>Mammary epithelial cells</td>
</tr>
</tbody>
</table>

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the target tissue of interest may not be related. For example, tea contains a number of polyphenols (catechins) including (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC) and (+) gallatechin. When the bioavailability, tissue distribution and biological activities of tea catechins were measured in rats, the levels of catechins in different tissues differed from those in plasma (38). For example, the large intestine and bladder had twice the concentrations and the liver had one eighth the concentration of tea catechins as plasma. When individual catechins were examined, the highest concentration of EGCG was detected in bladder and kidney, whereas the highest EGC was found in the large intestine and bladder had twice the concentrations and tissues differed from those in plasma (38). For example, the levels of catechins in different tissues were measured in rats, the levels of catechins in different tissues differed from those in plasma (38).

TABLE 1
Sources of exfoliated cells that can be collected in human studies

<table>
<thead>
<tr>
<th>Epithelial Cell</th>
<th>Biological sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>Urine</td>
</tr>
<tr>
<td>Buccal</td>
<td>Mouth rinse</td>
</tr>
<tr>
<td>Colon</td>
<td>Stool, gastric lavage</td>
</tr>
<tr>
<td>Lung</td>
<td>Sputum, bronchoalveolar lavage</td>
</tr>
<tr>
<td>Mammary</td>
<td>Nipple aspirate, ductal lavage, breast milk</td>
</tr>
<tr>
<td>Prostate</td>
<td>Semen</td>
</tr>
</tbody>
</table>

TABLE 2
Molecular analyses that have been conducted in exfoliated cells

<table>
<thead>
<tr>
<th>Molecular sample</th>
<th>Analysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Mutation</td>
<td>8–12</td>
</tr>
<tr>
<td>DNA</td>
<td>Amplification</td>
<td>13,14</td>
</tr>
<tr>
<td>DNA</td>
<td>Methylation</td>
<td>15,16</td>
</tr>
<tr>
<td>DNA</td>
<td>Strand breaks</td>
<td>17,18</td>
</tr>
<tr>
<td>DNA</td>
<td>Carcinogen-DNA adducts</td>
<td>19–21</td>
</tr>
<tr>
<td>DNA</td>
<td>Micronuclei formation</td>
<td>22–30</td>
</tr>
<tr>
<td>RNA</td>
<td>Gene expression</td>
<td>31,32</td>
</tr>
<tr>
<td>Protein</td>
<td>Protein expression</td>
<td>13,33</td>
</tr>
<tr>
<td>Protein</td>
<td>Enzyme activity</td>
<td>34,35</td>
</tr>
</tbody>
</table>
their combination decreased the formation of micronuclei in exfoliated cells of betel chewers (26). Other chemoprotective agents that have led to a decrease in micronuclei frequencies in oral mucosal cells of healthy individuals and/or patients with oral lesions include α-tocopherol, iotretinoin, and turmeric and related substances (22,27,28). Intervention studies with combined treatment of 15 mg retinol, 200 mg riboflavin and 50 mg zinc in Chinese subjects living in an area with a high incidence of esophageal cancer led to a reduction in the micronuclei frequency in esophageal cells (0.19% in the supplemented group vs. 0.31% in the placebo group) but not in oral cells (29). Green tea was protective against micronuclei formation in exfoliated oral cells of individuals with oral leukoplasia; this decrease was paralleled by a decrease in the frequency of both micronuclei and chromosomal aberrations in peripheral blood lymphocytes (30). After 6 mo of a mixed tea intervention, the micronuclei formation in exfoliated oral cells was reduced from 10.5 to 5.4/1000 cells, and the micronuclei of lymphocytes and chromosome aberration rate in the peripheral blood lymphocytes were reduced from 3.9 to 2.6/1000 cells and from 2.5 to 1.7/100 cells, respectively (30). In contrast, these values increased in placebo-treated controls. These results show that tea has a significant chemopreventive effect against DNA damage in exfoliated cells. Thus, the assay of micronucleated exfoliated cells has been utilized to study the protective effect of bioactive food components in a biological response with relevance to cancer, i.e., chromosomal breakage in target epithelial cells.

Future Directions. Future studies must evaluate the potential usefulness of more “state of the art” analyses in exfoliated cells in nutritional studies. Examples include microarray analysis for changes in gene expression and surface enhanced laser desorption ionization (SELDI) MS for protein expression. The National Cancer Institute is currently conducting two studies utilizing nipple aspirations. First, they are comparing protein expression via two-dimensional gels and SELDI MS from the breasts of women with different risks for breast cancer with the expression patterns in serum. It may be possible to define patterns of protein expression (fingerprints) that correlate with the risk of breast cancer. They are also collecting nipple aspirate fluid from high risk women who are being treated with the chemoprevention drug, difluoromethylornithine, both before and after drug administration to look for modulation of potential surrogate end points. Similar studies should be conducted looking at the effect of bioactive food components on many different types of exfoliated cells. Future studies are also required to determine whether dietary-induced changes in biomarkers that are measured in exfoliated cells are indicative of changes in target tissues or whether they are representative of global changes within the body.

In summary, exfoliated cells hold potential as a tool for monitoring human exposure and response to bioactive food components. Many sources of exfoliated epithelial cells can be obtained for lung, colon, mammary, bladder and buccal cells. Although bioactive food components have been shown to inhibit carcinogen-DNA adduct formation, DNA damage by the Comet assay and micronuclei formation in exfoliated cells, very few studies have utilized exfoliated cells to analyze exposure to bioactive food components. Future studies are warranted to determine the quality and quantity of exfoliated cells that can be obtained as well as the potential usefulness of exfoliated cell surrogates markers of exposure for cells in target tissues in nutritional intervention studies.

LITERATURE CITED


