Potential of using breast milk as a tool to study breast cancer and breast cancer risk

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Are carcinogenic man-made compounds accumulating in breast milk and compromising the health of women and children? Numerous studies have examined the relationship between pollutants and breast cancer, yet the role of environmental exposures in the etiology of breast cancer remains unclear. The association between breast cancer and 'pollutant load' is difficult to study because only small amounts of tumor and normal breast tissue are available for chemical analyses. Thus, in any individual breast tissue sample, only a few chemicals can be easily measured, while hundreds of exogenous compounds are known to be present. Although exposure assessment tools can help predict the levels of particular chemicals in the breast, there is no reasonable way to collect breast tissue for confirmation. Several investigators have suggested using nipple aspiration and ductal lavage to obtain breast fluid and epithelial cells; however, the inconvenience, discomfort, cost and small amount of fluid and cells retrieved make these procedures unsuitable for the type of large, prospective study that is needed to investigate the role of exposure to environmental pollutants in the etiology of breast cancer.

Sampling breast milk offers a unique opportunity to investigate the relationship between environmental exposures and breast cancer. In addition to the pollutants in the fat and aqueous fractions of the milk, millions of naturally exfoliated breast epithelial cells as well as immune system and blood cells are present, providing biomarkers of both exposure and effect. The epithelial cells, potentially originating from any gland in the breast, can be separated from the other cells in the milk and examined for genetic, epigenetic and gene-expression changes associated with pollutant exposure.

Since the discovery of polychlorinated biphenyls (PCBs) and organochlorine pesticides in breast milk in the early 1970s, there has been mounting concern that accumulation of pollutants in breast tissue may increase a woman's risk of developing breast cancer. Today, there is no doubt that breast milk is a rich source of environmental pollutants, as chemical analyses have revealed the presence of most man-made compounds in human milk. While environmental contaminants from personal care, pharmaceutical, household and industrial products have all been detected in human milk (e.g., titanium dioxide, triclosan, synthetic musks, lamotrigine, bisphenol A, polychlorinated biphenyls, dibenzofurans and dibenzodioxins, pesticides, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and perfluorinated compounds), most studies have examined just one or two classes of compounds. Indeed, the tens of thousands of potential pollutants in breast milk make it unrealistic to attempt to measure all, or even most, of them.

Adding to the complexity of the mixture of exogenous chemicals present in human milk is the fact that not all compounds of even a single 'group' have the same effect. A classic example is that of PCBs. Some PCBs are 'dioxin-like' in their activity, acting through the aryl hydrocarbon receptor, while other PCBs are estrogenic, acting through the estrogen receptor. Since exposure to estrogens increases the risk of developing breast cancer, and PCBs were known to have estrogenic activity and accumulate in fatty tissue, it was hypothesized that PCB levels would be higher in breast tumor tissue than in normal breast tissue. To test this hypothesis, individual PCB congeners known to have estrogenic activity, as well as those congeners known to induce

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the cytochrome P450 enzymes that metabolize 17β-estradiol, thus inactivating it, need to be measured. While early studies tended to report the total level of PCBs in breast milk, more recent studies provide individual levels for as many as 101 of the 203 possible PCB congeners. However, to fully test the 'estrogen hypothesis', the levels of all estrogenic compounds in the breast should be measured. This is a daunting task, as a number of the pesticides, plasticizers and synthetic musks present in human milk are reported to have weak estrogenic activity. One approach being used to account for the multiple chemicals, with their potentially opposing effects, is to measure the activity detected in milk. For example, in a few studies, extracts of human milk have been tested in reporter assays modified to detect estrogen receptor- and aryl hydrocarbon receptor-dependent transcriptional activity.

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A complementary approach to analyzing the specific activity of milk extracts is to assess the effects of chemical exposure on the cells in breast milk. Several studies have examined human milk cells for DNA damage using the micronuclei or Comet assay, and a correlation between DNA damage and extract toxicity has been demonstrated. However, in general, the variability of DNA damage observed among cells within a single milk sample is extremely high, obscuring potential relationships between DNA damage and environmental or physiologic variables. The high variability among samples is partly due to the mixed population of cell types present in human milk; therefore, using a single cell type might greatly reduce this variability. Epithelial cells represent approximately 10–30% of the total cells expressed in human milk, and viable mammary epithelial cells are easily isolated using antibody-coated magnetic beads. RNA and DNA can be isolated from the epithelial-enriched cell populations, providing the ability to measure changes in gene expression, as well as genetic and epigenetic pollutant-related aberrations.

Possibly the most promising biomarker of breast cancer risk is methylation in the promoter regions of specific genes. Methylation is a type of epigenetic change; a nonsequence modification of DNA linked to overall changes in gene expression. Methylation within a cytosine

guanine dinucleotide-rich area (CpG island) of the promoter region of a gene that is normally unmethylated is correlated with silencing of that gene. At least 40 genes involved in tumor suppression, cell-cycle control, DNA repair or toxicant metabolism have been found to be silenced by promoter hypermethylation in breast cancers. Increased promoter hypermethylation is one of the most common molecular changes detected in breast cancer, as the silencing of genes involved in cell-cycle control, DNA repair, tumor suppression and metabolism of toxicants greatly increases the chances that a normal cell will acquire the characteristics needed to become a cancer cell. Environmental exposures and diet are thought to alter our methylation patterns as we age, and *in vitro* exposure of breast cells to estrogens has resulted in increased methylation of tumor suppressor genes. Thus, methylation, as well as other types of epigenetic and genetic analyses from epithelial cells, allows the possibility of assessing damage that may be:

- Independent of other measures
- Part of a causal pathway involving chemical exposures
- Mediated by socio–demographic factors
- Potentially diagnostic of future morbidity such as breast cancer

The additional collection of in-depth personal survey information raises the possibility of linking behavioral, dietary and family history information to epigenetic models relating risk factors with the presence of specific chemicals, the presence of genetic damage or, plausibly, an entire causal chain from exposures through chemical agents, impacting genetic damage and, in prospective follow-up, relating to eventual morbidity outcomes.

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Another complex set of potential interactions are also open to breast milk research in the potential for intergenerational studies that link the pathways described previously to longer term follow-up and bioassay of breastfed children. There are some disadvantages to breast milk bioassays. Breast milk does not offer the easy genetic assay of saliva kits and requires handling similar to serum samples. In addition,

most importantly, it is a sample largely limited by gender and life stage. Nonetheless, breast milk can provide, at a juncture of generations, a critical depth of information of unique potential for oncological research.

It is not always the case that there is a strong apparent tie between detailed laboratory investigations of genetic material and more general policy concerns of social epidemiology. However, the unique potential of breast milk for studies that investigate pathways involving specific chemical compounds and potential morbidity consequences makes it a particularly useful bioassay for researchers interested in such diverse general health topics as environmental justice and the social distribution of risks, the impact of cultural and ethnic differences in health-related behaviors and diets, and consumption behaviors and product safety. In the majority of such studies, research has been limited to examining the difference between individual characteristics and a presumed exposure to risk (e.g., consumption of hazardous products and proximity to hazardous sites). Advances in addressing such concerns have been made recently with an increasing use of genetic bioassays, although such studies are still relatively rare in social epidemiology. Ideally, future research would not only link socio–demographic and behavioral differences in a population with concrete measures of resulting differences in exposures and accumulation of potentially harmful or hazardous substances, but also assess whether such exposures have a demonstrable impact on methylation or other measurements of genetic damage, as well as leaving a potential for ultimate morbidity follow-up. Breast milk provides such an opportunity.

Oncology bears a shared, but perhaps particularly acute, social responsibility in conducting and presenting research. Given the rapid dissemination of information in the current environment and the critical attention given by both the public and individuals with personal interests related to cancer and cancer research, oncological research is an example of what social scientists might refer to as a form of 'public epidemiology'. Breast milk research, with the additional attention it can receive from nursing or prospective mothers, advocacy groups and even the press, bears an additional burden to assure that the dissemination of research with intimidating reference to chemical compounds, genetic damage and cancer or other morbidities, does not generate a false impression with the public that associates negative constructs with the clearly beneficial practice of breastfeeding infants.

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