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Analysis of unconventional approaches for the rapid detection of surface lectin binding ligands on human cell lines

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Summary

For over a decade our laboratory has developed and used a novel histochemical assay using derivatized agarose beads to examine the surface properties of various cell types. Most recently, we have used this assay to examine lectin binding ligands on two human cell types, CCL-220, a colon cancer cell line, and CRL-1459, a non-cancer colon cell line. We found that CCL-220 cells bound specific lectins better than CRL-1459, and this information was used to test for possible differential toxicity of these lectins in culture, as a possible approach in the design of more specific anti-cancer drugs. Although we have examined the validity of the bead-binding assay in sea urchin cell systems, we have not previously validated this technique for mammalian cells. Here the binding results of the bead assay are compared with conventional fluorescence assays, using lectins from three species (*Triticum vulgaris*, *Phaseolus vulgaris*, and *Lens culinaris*) on the two colon cell lines. These lectins were chosen because they seemed to interact with the two cell lines differently.

Binding results obtained using both assays were compared for frozen, thawed and fixed; cultured and fixed; and live cells. Both qualitative and quantitative fluorescence results generally correlated with those using the bead assay. Similar results were also obtained with all of the three different cell preparation protocols. The fluorescence assay was able to detect lower lectin binding ligand levels than the bead assay, while the bead assay, because it can so rapidly detect cells with large numbers of lectin binding ligands, is ideal for initial screening studies that seek to identify cells that are rich in surface binders for specific molecules. The direct use of

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frozen, thawed and fixed cells allows rapid mass screening for surface molecules, without the requirement for costly and time consuming cell culture.
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Introduction

Over the past decade, this laboratory has developed and used a novel histochemical assay to examine cell surface properties (Heinrich et al., 2005; Khurrum et al., 2002; Navarro et al., 2002; Salbilla et al., 1999; Latham et al., 1995a, b; Latham and Oppenheimer, 1999). This technique involves mixing various cell types to be studied separately with agarose beads derivatized with about 100 different amino acids, sugars, lectins and other proteins, nucleotides and other molecules. Most histochemical assays use stains to identify the biochemical components of cells and tissues. The bead assay uses derivatized beads to do the same thing. If a cell adheres to a bead derivatized with a specific lectin and the adhesion is blocked by the lectin's preferential binding sugar, such results would indicate that a ligand for that lectin is present on the cell surface.

Many past studies have used derivatized beads to examine cell surface properties (Aketa et al., 1979; Iwig et al., 1980; Takahashi and Tavassoli, 1981; Nenci, 1983; Pincus, 1984; Johnson and Silver, 1989; Matsuoka and Tavassoli, 1989a, b; Hayes et al., 1990; Kijimoto-Ochiai and Uede, 1995), but we are unaware of any studies, other than ours, that use so many different beads to rapidly assay cell surface properties (Khurrum et al., 2002; Navarro et al., 2002; Salbilla et al., 1999; Latham and Oppenheimer, 1999; Latham et al., 1995a, b; Heinrich et al., 2005). Previous studies from our laboratory have involved many unconventional approaches. For example, previously frozen cells that were fixed with formaldehyde were used instead of freshly cultured cells (Khurrum et al., 2002). Fixation did not alter authentic surface properties (Navarro et al., 2002). In some cases we used live cells in their physiological medium (Latham et al., 1995a, b; Navarro et al., 2002).

Previously experiments were conducted to examine the validity of the bead-binding assay using sea urchin embryos and fluorescent (FITC) labeled lectins (Latham et al., 1995a, b). When lectin beads bound to the cells, the FITC lectins also bound. When the lectin beads did not bind, neither did the FITC labeled lectins bind (Latham et al., 1995a, b). In both cases, hapten sugars inhibited lectin binding to the cells. These results led to the conclusion that the bead assay was a valid approach to examining cell surface properties, such as lectin binding ligands, on the cell types tested.

Live sea urchin cells in sea water, however, do not present the same sort of challenge as other cell types under other conditions. Because of the usefulness and rapidity of the bead assay, it is important to examine its validity with other cell types under other conditions. In this study, we examine lectin binding ligands on two human colon cell lines: CCL-220, a colon cancer cell line, and CRL-1459, a non-cancer colon cell line, using both the bead and conventional fluorescence assays. We also examined binding using both assays and three cell preparation protocols: (1) frozen, thawed and fixed cells assayed in distilled water, (2) cultured and fixed cells assayed in distilled water, and (3) live cells assayed in saline.

The main purpose and novelty of this study, therefore, is to determine if the conventional fluorescence assay gives similar results as the unconventional bead assay, as a means of validating the use of the bead assay in mammalian cell systems. In addition, the study explores which cell preparation procedure is best for the most rapid detection of specific cell surface molecules.

Material and methods

Cell lines

Two human cell lines, colon cancer cells tumorigenic in nude mice (CCL-220/Colo320DM) and non-cancerous colon cells (CRL-1459/CCD-18Co), were obtained frozen from the American Type Culture Collection (ATCC, Manassas, VA). In some experiments the frozen ATCC cells were used directly. In those experiments, cells were thawed in a 37 °C water bath for 40–60 s, washed by centrifugation for 3 min at 1000*g* in 1.5 ml Dulbecco's phosphate buffered saline with calcium and magnesium chloride (PBS; Sigma, St. Louis, Missouri, USA), and fixed in 1% formaldehyde in PBS for at least 30 min. Prior to use, the fixed cells were washed thrice by centrifugation at 1000*g* for 3 min in 1.5 ml of distilled water. Cell suspensions were then diluted by adding 8.5 ml of distilled water yielding

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