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A new impedance based approach to test the activity of recombinant protein – Semaphorins as a test case

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ABSTRACT

The biological activity of a recombinant protein is routinely measured using a bioassay such as an enzyme assay. However, many proteins have no enzymatic activity and in many cases it is difficult to devise a simple and reliable approach to test their activity. Semaphorins, Ephrins, Slits, Netrins or amylin-assisted proteins have numerous activities affecting many systems and cell types in the human body. Most of them are also able to induce rapid cytoskeleton changes at least in some cell types. We assumed therefore, that such proteins might be tested based on their ability to modulate the cytoskeleton. Here we tested a number of semaphorins in an impedance based label-free platform that allows for dynamic monitoring of subtle morphological and adhesive changes. This system has proved to be a very fast, sensitive and effective way to monitor and determine the activity of such proteins. Furthermore we showed that it is possible to customize a cell-protein system by transfecting the cells with specific receptors and test the cell response following the addition of the recombinant ligand protein. Since other protein families such as Ephrins and Netrins can also influence the cytoskeleton of some cells, this approach may be applicable to a large number of proteins.

1. Introduction

The production of a recombinant protein is a critical step in studying the function of proteins. However in many cases there are no simple methods to determine the activity of the recombinant protein. One such example is the semaphorin proteins. The semaphorin family is comprised of 20 proteins with an important role in a wide range of biological processes across several organ systems including neural circuit assembly, bone formation, kidney and heart development, angiogenesis, immune response and cancer biology (Capparuccia and Tamagnone, 2009; Takamatsu and Kumanogoh, 2012; Yazdani and Terman, 2006). Semaphorins commonly act as ligands that bind directly to plexins or neuropilins (Capparuccia and Tamagnone, 2009). Although the importance of these molecules is clear there is no simple and fast method to determine their activity. To date there are two approaches to test semaphorin activity. The first method is to directly test the purified protein in a specific assay. However, it is impossible to know if a protein is not active because its activity was lost along the purification process or because the particular assay is inappropriate. A second method is based on the ability of the vast majority of semaphorins to induce cytoskeletal and morphological changes in different cell types (as long as they express the correct combination of receptors for the specific semaphorin). The most used approach is called the COS-7 cell collapse assay (Turner and Hall, 2006). In this assay COS-7 cells are transfected with the specific receptors for the tested proteins. Then, 48–72 h after transfection cells are challenged with the appropriate semaphorin and one-hour later cells are fixed, labeled and analyzed for morphological changes. Although it is possible to use this morphological assay to monitor semaphorin activities this method is slow and labor intensive. Moreover, in many cases it is difficult to determine whether the cell responded or not due to the variability of morphologies among the COS-7 cells.

The impedance based approach utilizes an electronic readout to non-invasively quantify cellular status in real-time (Ge et al., 2009). In this system cells are seeded onto specialized plates, which are integrated with microelectronic sensor arrays. The interaction of cells with the microelectrode surface generates a cell-electrode impedance response, which correlates with the number of the cells present in the well. Monitoring over time is used to track cell proliferation, cell death and even cell migration in real time (Diemert et al., 2012; Ge et al., 2009; Kustermann et al., 2013; Rahim and Uren, 2011; Takamatsu and Kumanogoh, 2012). However, cell-electrode impedance response is likely to be sensitive to

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cell morphology and adherence. Although the impedance assay is sensitive to changes in cell number we assume we could use it to test protein-induced cytoskeletal changes as long as the changes that are monitored are rapid and can be seen within minutes. Semaphorins are known to induce rapid and robust cytoskeletal changes that affect cell morphology and adhesion in different type of cells (Tran et al., 2007). We have therefore postulated that impedance-based systems can be used to monitor the activity of semaphorins in a rapid and reliable way. Since other protein families such as ephrins can also induce such cytoskeletal changes, this method may be applicable to even larger numbers of proteins.

2. Materials and methods

2.1. Semaphorin preparation

Media conditioned by HEK293 cells, which were transiently transfected with Sema3A, Sema3F, Sema3B, Sema6A-Fc, Sema4B-Fc, Fc-only or non-transfected cells were collected. All semaphorins included a myc tag. Concentrations of the Fc fusion proteins were evaluated by western blot with anti-hIgG antibody. By using the myc tag (which was presented on all proteins), we valuated the concentrations of semaphorins that contained only the myc tag but not Fc).

2.2. COS-7 cell contraction assay

A COS-7 cell contraction assay was carried out essentially as described in (Ben-Zvi et al., 2007), with minor modifications. COS-7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Cells were transfected with 1000 ng DNA. In all experiments, cells were transfected with expression vectors for pEGFP (200 ng) and PlexinB1 (800 ng). *Trans*IT-X2® Dynamic Delivery System (Mirus) was used as a transfection reagent in all experiments. After 48 h of transfection, 36 fields with cells were photographed (using GFP channel) before the addition of Sema4D-Fc or Fc-only control. Using scanning stage apparatus, the same 36 fields were photographed 40 min later. Cell images were captured using a fluorescence microscope (Olympus, Hamburg, Germany) equipped with a cooled CCD camera

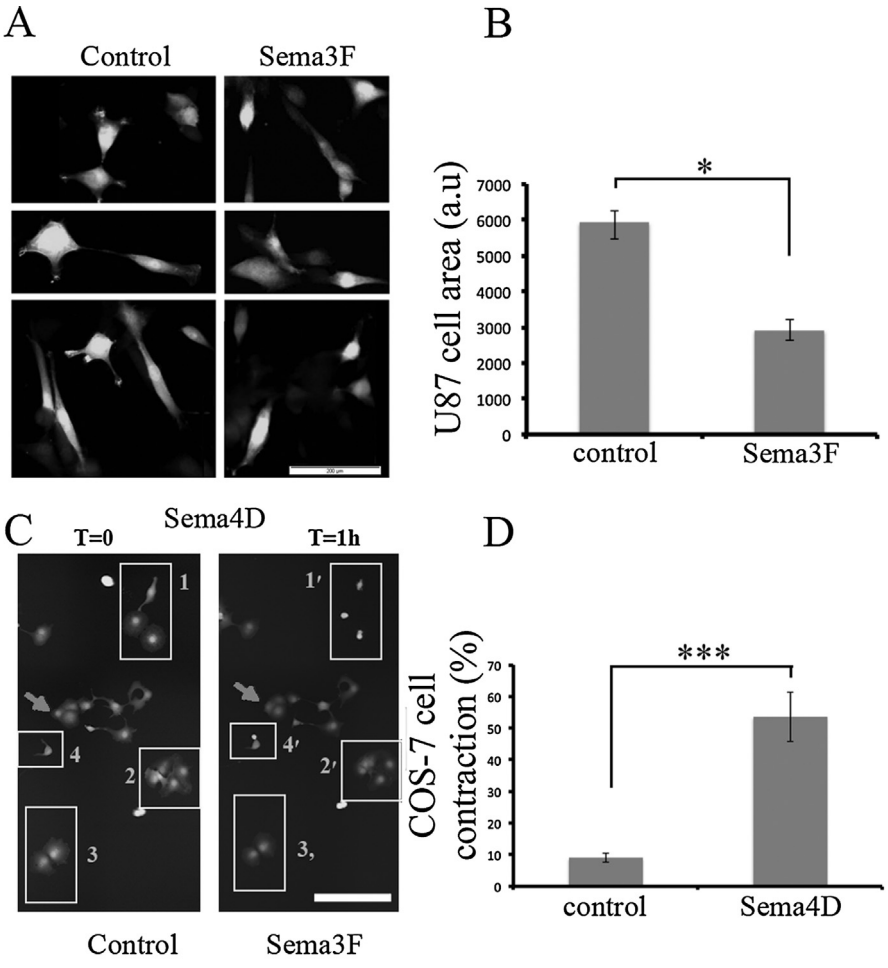

Fig. 1. semaphorin induced morphological responses of U87 and COS-7 cells. (A and B) U87 cells were infected with GFP expressing lentivirus and treated with control or 120 ng/ml Sema3F for 60 min following by fixation. 30 random fields for each treatment where photographed (representative cells are shown in (A). Scale bar 200 μM. Cell size was estimated using image J measurement of all cells in each of the photographed fields. The cell size measurements presented in arbitrary units (a.u) are presented (B). C, D. COS-7 cells were transfected with GFP and PlexinB1 expression vectors as indicated in the methods section. GFP expressing cells were photographed immediately before and 40 min after the treatment with Sema4D-Fc or Fc-only control supernatant (C). By monitoring only the GFP expressing cells we focused only on positively transfected cells. Since we used a ratio of 5:1 in favor of the PlexinB1 it is likely that most GFP expressing cells are also expressing PlexinB1. However the morphological changes shown by different cells are vastly different and therefore make the scoring of response very labor intensive and somewhat subjective. Examples of the different morphologies in the same filed are shown in white boxes and marked with numbers: Box 1: example of a dramatic morphological effect of a few cells. Box 2: although cells are likely to express the receptor no change in cell aria is detected (3% reduction). Box 3: cell aria is somewhat changed (reduced by 30%). However while visually scoring hundreds of cells it can be easily missed. Box 4: morphological change can be detected but there is almost no change in cell aria. Therefore if using automated cell area measurement these responses will be missed. Arrowheads point to a cell in which aria was increased slightly instead of decreasing (18%). Scale bar is 100 μM. The results are means ($n = 3$) ± s.e.m.