INTRODUCTION

The emergence of an increasing number of immunotherapy biomarkers and the importance of immune checkpoints within the tumor microenvironment has resulted in a need for high-plex immunohistochemistry (IHC) assays. Using highly specific and validated antibodies developed for this purpose, we constructed several fluorescent multiplexed, TSA-based assays to examine the frequency, spatial localization, and proximity of immune cells within the tumor microenvironment. Our data demonstrates the feasibility of simultaneous detection of seven fluorochromes in order to visualize immunosuppressive receptors associated with the exhausted T cell phenotype, myeloid-derived suppressor cells, and the PD-1:PD-L1 axis. Our findings demonstrate the utility of multiplex IHC to deconvolute protein expression and interactions within the complex tumor microenvironment.

METHODS

TSA was used to serial stain tumor tissue of various types. This protocol allows for the use of multiple rabbit monoclonal antibodies in a single panel. A Mantra quantitative pathologic workstacion (PerkinElmer) was used to spectrally unmix the fluorescent signal in each image, and the InForm Image Analysis software (PerkinElmer) was used to provide quantitative data. Immuno- and co-localization panels have been constructed, as well as those that focus on receptors involved in certain targeted cancer therapies.

CONCLUSIONS

- Multiplex IHC panels consisting of up to six targets plus DAPI were constructed and validated in various tumor types.
- Highly detailed images illustrating the utility of mIH to detect:
  - Spatial localization of immune cells within the tumor microenvironment.
  - Co-localization and frequency of immune checkpoint receptors.
  - Proximity of suppressive immune cells and immune checkpoints indicative of receptor-ligand interactions.
- mIH may provide a more in-depth understanding of the role of suppressive immune cells and their interactions with tumor cells in the process of immune evasion.
- Any Cell Signaling Technology, Inc. IHC-validated antibody can be used to construct mIH panels.

Highly Multiplexed IHC Assays to Examine Immune Checkpoints and Biomarkers for Immunotherapy

INTRODUCTION

The emergence of an increasing number of immunotherapy biomarkers and the importance of immune checkpoints within the tumor microenvironment has resulted in a need for high-plex immunohistochemistry (IHC) assays. Using highly specific and validated antibodies developed for this purpose, we constructed several fluorescent multiplexed, TSA-based assays to examine the frequency, spatial localization, and proximity of immune cells within the tumor microenvironment. Our data demonstrates the feasibility of simultaneous detection of seven fluorochromes in order to visualize immunosuppressive receptors associated with the exhausted T cell phenotype, myeloid-derived suppressor cells, and the PD-1:PD-L1 axis. Our findings demonstrate the utility of multiplex IHC to deconvolute protein expression and interactions within the complex tumor microenvironment.

METHODS

TSA was used to serial stain tumor tissue of various types. This protocol allows for the use of multiple rabbit monoclonal antibodies in a single panel. A Mantra quantitative pathologic workstacion (PerkinElmer) was used to spectrally unmix the fluorescent signal in each image, and the InForm Image Analysis software (PerkinElmer) was used to provide quantitative data. Immuno- and co-localization panels have been constructed, as well as those that focus on receptors involved in certain targeted cancer therapies.

CONCLUSIONS

- Multiplex IHC panels consisting of up to six targets plus DAPI were constructed and validated in various tumor types.
- Highly detailed images illustrating the utility of mIH to detect:
  - Spatial localization of immune cells within the tumor microenvironment.
  - Co-localization and frequency of immune checkpoint receptors.
  - Proximity of suppressive immune cells and immune checkpoints indicative of receptor-ligand interactions.

mIH may provide a more in-depth understanding of the role of suppressive immune cells and their interactions with tumor cells in the process of immune evasion.

Any Cell Signaling Technology, Inc. IHC-validated antibody can be used to construct mIH panels.

Highly Multiplexed IHC Assays to Examine Immune Checkpoints and Biomarkers for Immunotherapy

INTRODUCTION

The emergence of an increasing number of immunotherapy biomarkers and the importance of immune checkpoints within the tumor microenvironment has resulted in a need for high-plex immunohistochemistry (IHC) assays. Using highly specific and validated antibodies developed for this purpose, we constructed several fluorescent multiplexed, TSA-based assays to examine the frequency, spatial localization, and proximity of immune cells within the tumor microenvironment. Our data demonstrates the feasibility of simultaneous detection of seven fluorochromes in order to visualize immunosuppressive receptors associated with the exhausted T cell phenotype, myeloid-derived suppressor cells, and the PD-1:PD-L1 axis. Our findings demonstrate the utility of multiplex IHC to deconvolute protein expression and interactions within the complex tumor microenvironment.

METHODS

TSA was used to serial stain tumor tissue of various types. This protocol allows for the use of multiple rabbit monoclonal antibodies in a single panel. A Mantra quantitative pathologic workstacion (PerkinElmer) was used to spectrally unmix the fluorescent signal in each image, and the InForm Image Analysis software (PerkinElmer) was used to provide quantitative data. Immuno- and co-localization panels have been constructed, as well as those that focus on receptors involved in certain targeted cancer therapies.

CONCLUSIONS

- Multiplex IHC panels consisting of up to six targets plus DAPI were constructed and validated in various tumor types.
- Highly detailed images illustrating the utility of mIH to detect:
  - Spatial localization of immune cells within the tumor microenvironment.
  - Co-localization and frequency of immune checkpoint receptors.
  - Proximity of suppressive immune cells and immune checkpoints indicative of receptor-ligand interactions.

mIH may provide a more in-depth understanding of the role of suppressive immune cells and their interactions with tumor cells in the process of immune evasion.

Any Cell Signaling Technology, Inc. IHC-validated antibody can be used to construct mIH panels.

Highly Multiplexed IHC Assays to Examine Immune Checkpoints and Biomarkers for Immunotherapy

INTRODUCTION

The emergence of an increasing number of immunotherapy biomarkers and the importance of immune checkpoints within the tumor microenvironment has resulted in a need for high-plex immunohistochemistry (IHC) assays. Using highly specific and validated antibodies developed for this purpose, we constructed several fluorescent multiplexed, TSA-based assays to examine the frequency, spatial localization, and proximity of immune cells within the tumor microenvironment. Our data demonstrates the feasibility of simultaneous detection of seven fluorochromes in order to visualize immunosuppressive receptors associated with the exhausted T cell phenotype, myeloid-derived suppressor cells, and the PD-1:PD-L1 axis. Our findings demonstrate the utility of multiplex IHC to deconvolute protein expression and interactions within the complex tumor microenvironment.

METHODS

TSA was used to serial stain tumor tissue of various types. This protocol allows for the use of multiple rabbit monoclonal antibodies in a single panel. A Mantra quantitative pathologic workstacion (PerkinElmer) was used to spectrally unmix the fluorescent signal in each image, and the InForm Image Analysis software (PerkinElmer) was used to provide quantitative data. Immuno- and co-localization panels have been constructed, as well as those that focus on receptors involved in certain targeted cancer therapies.

CONCLUSIONS

- Multiplex IHC panels consisting of up to six targets plus DAPI were constructed and validated in various tumor types.
- Highly detailed images illustrating the utility of mIH to detect:
  - Spatial localization of immune cells within the tumor microenvironment.
  - Co-localization and frequency of immune checkpoint receptors.
  - Proximity of suppressive immune cells and immune checkpoints indicative of receptor-ligand interactions.

mIH may provide a more in-depth understanding of the role of suppressive immune cells and their interactions with tumor cells in the process of immune evasion.

Any Cell Signaling Technology, Inc. IHC-validated antibody can be used to construct mIH panels.