Screening method for estrogenic and anti-estrogenic activity

Background information concerning the patent applications
EP 3517967 A1
WO 2019145517 A1

The following sections describe the patent application status and potential patent exploitation scenarios of the E-Morph Assay. Scientific publications reporting on the described findings are currently in preparation.

Interested licensees may contact 9@bfr.bund.de for further information about the E-Morph Assay or possible licensing scenarios.

Patent application status:

1) Regional application: European Patent Office (EPO)

   https://data.epo.org/gpi/EP3517967A1

   a. First filing:
      26 Jan 2018
      EP 18153664
   b. Search report available:
      16 Aug 2018
   c. Publication of patent application:
      31 Jul 2019
      EP 3517967 A1
   d. Examination in progress

2) International application (PCT):

   https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2019145517

   a. PCT filing:
      25 Jan 2019
      EP 2019051920
   b. PCT search report available:
      17 Apr 2019
   c. Publication of patent application:
      01 Aug 2019
      WO 2019145517 A1
   d. National applications in preparation

Potential patent exploitation scenarios:

1) Safety of industrial chemicals, biocides and pesticides

The E-Morph Assay may complement toxicological testing strategies to identify and characterize estrogenic and anti-estrogenic activity of test chemicals for regulatory purpose to ensure consumer health protection.

Man-made chemicals in our environment, food or consumer products that interfere with the hormone (endocrine) system in humans, e. g. the estrogen signaling pathway, and eventually lead to adverse health effects, e. g. cancer formation, are of high concern and their production and marketing is subject to specific legislative obligations worldwide.

Thus, corresponding national legislation stipulates within the framework of consumer health protection that environmental chemicals must be tested for their potential to trigger adverse health effects. Especially, industrial chemicals, biocides and plant protection products have to be screened for their potential to interfere with the endocrine system eventually leading to adverse effects in humans and wildlife. Hereby it is also important to understand their dose-response.

In this context, it is essential to identify chemicals for their potential to develop an estrogenic effect.

E-cadherin is an adhesion molecule that is required for maintaining cell-cell contacts between epithelial cells. A disruption of these cell-cell contacts can lead the loss of cell adhesion between breast cells and the development of breast cancer. We discovered for the first time a new and specific estrogen receptor alpha-dependent effect that alters cell-cell contacts in breast cancer cells leading to a cellular redistribution of E-cadherin.

This finding led to the development of a novel and unique high throughput (HT)-compatible *in vitro* assay, the E-Morph Assay, for identification and characterization of chemicals with estrogenic activity by high content (HC)-based phenotypic screening. This assay specifically focuses on the estrogen receptor alpha-dependent regulation of E-cadherin-based cell-cell contact organization in an MCF-7 breast cancer cell line, which provides a novel endpoint with functional relevance.

2) Drug development

The E-Morph Assay may be applicable in the pharmaceutical sector, e. g., for (pre-) screening of novel active substances during the drug discovery process as well as the development and testing of pharmaceutical compounds, e. g. for treatment of patients with estrogen-receptor positive breast cancer.

Estrogens are essential hormones of the human endocrine system and involved in the regulation of diverse physiological functions ranging from development to reproduction and behavior. However, in a pathological setting, estrogens have also been implicated in stimulating uncontrolled cell proliferation and hormone-responsive tumor formation. Most metastatic breast cancers are invasive ductal carcinomas (IDC) that emerge in a stepwise fashion from the mammary epithelium lining the lactiferous duct. Many lines of evidence from basic to clinical research suggest the estrogen receptor (ER) signaling pathway to be of central importance for this process.

Therefore, anti-estrogenic pharmaceuticals are widely used in endocrine therapy of ER-positive breast cancers to block the activity of endogenous estrogens through different modes-of-action. Selective ER modulators (SERM) such as Tamoxifen and Raloxifene compete with endogenous estrogens from binding to their receptor, thereby reducing estrogen signaling activity. Selective ER degraders (SERD) such as Fulvestrant however fully block estrogen signaling activity and additionally reduce available protein levels of ERs. The choice between the use of SERMs or SERDs in breast cancer therapy mostly depends on cancer type, patient age and course of disease.

In addition to the detection of estrogenic substances activating the ER signaling pathway, the E-Morph Assay moreover also allows the identification and characterization of substances that block estrogen signaling. To do this, the E-Morph Assay uses the same experimental setup and functionally-relevant endpoint as described before, i. e. E-Cadherin membrane organization, and can be run on the same HC/HT screening pipeline.

The possibility to screen for both activating and inhibitory effects within a single *in vitro* assay in a HC/HT screening setup demonstrates the versatility and applicability of the E-Morph Assay.

3) Cancer diagnosis and treatment

A detailed characterization of cell membrane organization might be an instructive biomarker for the characterization of tumor progression and metastatic potential, and the analysis of therapeutic efficacy of known and novel pharmaceutical substances.

In clinical practice, the presence or absence of E-Cadherin membrane staining is a commonly used, but with regard to the heterogeneity of tumors and spatial organization of adherens junctions, not always straightforward biomarker for breast cancer classification.

Our analysis of breast cancer tissue sections from patients with diagnosed hormone-responsive metastatic breast cancer revealed that in some tumors the organization of adherens junctions is reminiscent of the effects of anti-estrogens in the E-Morph Assay. In this assay, interference with estrogen signaling activity also influenced cell adhesion, cell motility and cell stiffness *in vitro*, representing functional consequences that generally correlate with cancer progression.

Future studies using a greater diversity of breast cancer sections and considering patient data will be needed to further substantiate a potential correlation between E-Cadherin membrane distribution and breast cancer progression. However, our data already strongly suggests that the detection of E-Cadherin expression itself, as currently performed in clinical practice, might not be a sufficient biomarker for breast cancer classification and analysis. Rather, a detailed characterization of E-Cadherin localization might be much more instructive for the characterization of tumor progression and metastatic potential.

About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. It advises the German federal government and German federal states ("Laender") on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.