Contraceptive progestins differ in ability to stimulate proliferation of the human breast epithelium

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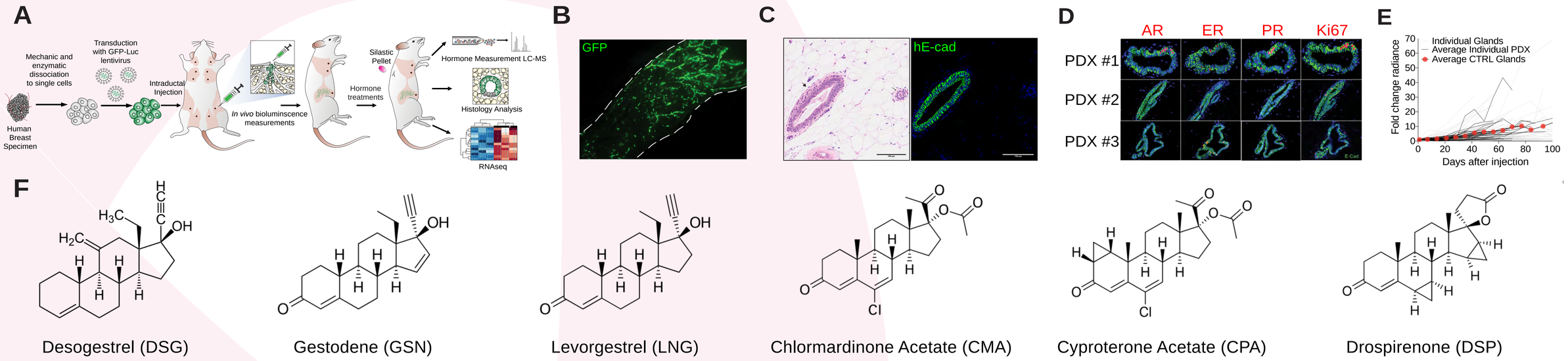
INTRODUCTION

Hormonal contraception exposes women to different progestins, transiently increasing breast cancer risk. However, how progestins affect the breast epithelium and whether individual progestins differentially affect cell proliferation in the normal human breast epithelium remain poorly understood. We hypothesized that individual progestins differentially affect cell proliferation of human breast epithelial cells (HBEC) and breast cancer risk.

EXPERIMENTAL SETTINGS

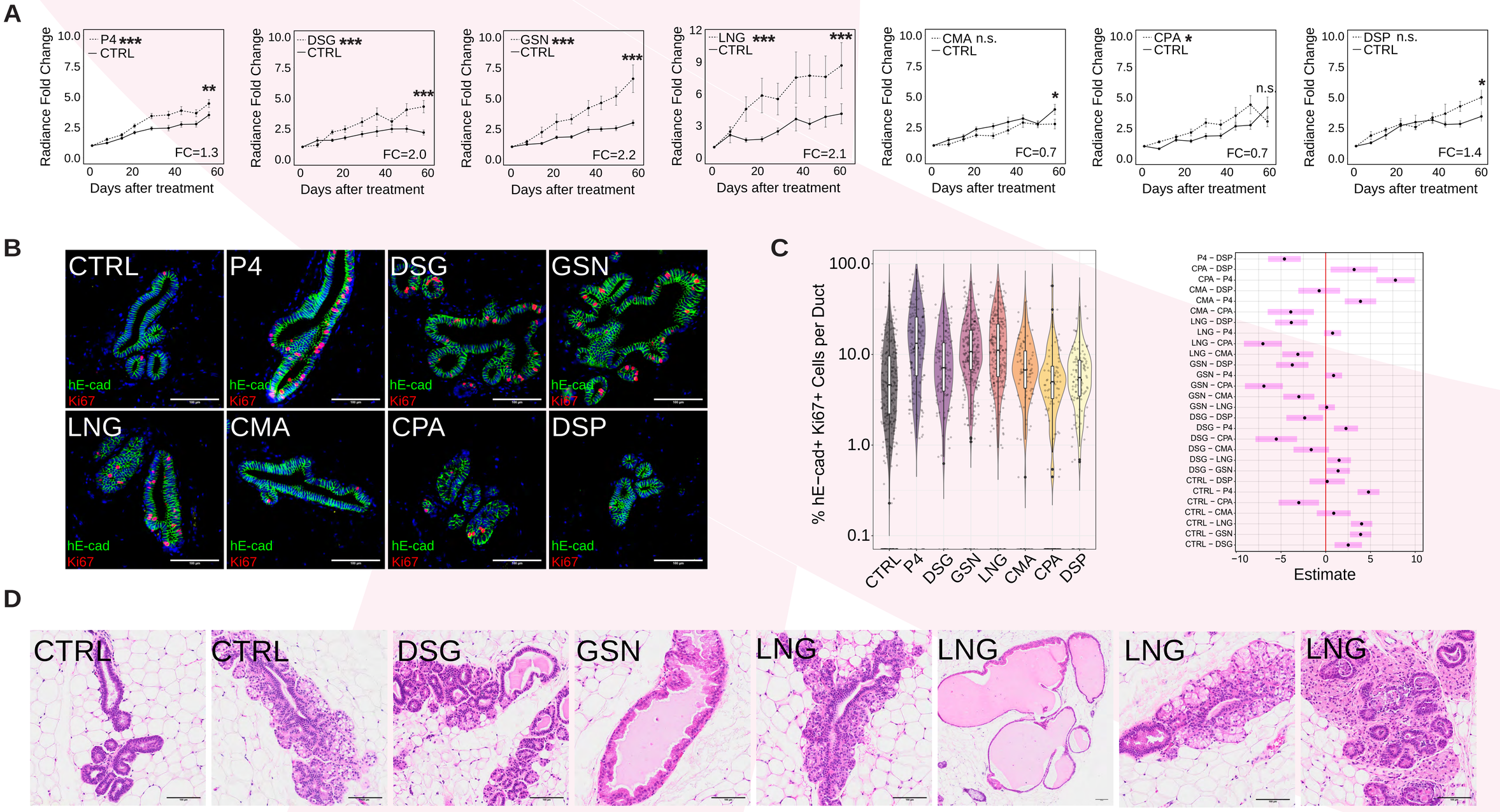
The Mouse INtraDuctal (MIND) model supports growth, hormone receptor expression and hormone responsiveness of HBECs *in vivo*. We tested the effects of 6 commonly used progestins in Switzerland on the xenografted HBECs.



(**A**) Experimental scheme of the MIND approach. (**B**) Fluorescence stereo micrograph of inguinal mammary gland 10 weeks after injection of GFP-labelled HBECs. (**C**) Left, H&E-stained section of xenografted gland 83 days after injection showing HBECs that distend a mouse duct (big arrow), small mouse ducts are shown (small arrow). Right, fluorescence micrograph of histological section of the same xenografted mammary duct stained with DAPI and immunofluorescence with anti hE-cad. (**D**) Immunofluorescence for AR, ER, PR and Ki67. (**E**) Spaghetti plot showing fold change radiance over time. (**F**) Molecular structure of the six drugs selected in our study, representative of the 4 different generations of progestins.

RESULTS

Progestins commonly used in Switzerland differ on their ability to promote proliferation and early precursor lesions of malignancy.



(**A**) Line charts showing in vivo growth of HBECs as measured by radiance after implantation of either control or different progestin-containing pellets. Points show means of radiance in individual glands ± SEM. Statistical significance was tested by fitting a mixed effect linear model with random effects to the log10-transformed data. Student's t-test or Wilcoxon rank-sum test was applied on log-transformed fold change values at endpoint. (**B**) Representative fluorescence micrographs showing co-IF with anti-Ki67 (red), and anti-Ecad (green) of xenografted milk ducts after 21 days treatment with vehicle or different progestins. (**C**) Left, violin plot showing the percentage of Ki67+ and hE-cadherin+ cells on total hEcadherin+ cells. Boxplots inside violins represent the interquartile range. Right, estimated marginal means are shown with their 95% confidence intervals. (**D**) Representative H&E-stained section of xenografted HBECs after 8 months of either vehicle or progestin treatments.

CONCLUSIONS

We developed a new preclinical model for the study of hormone receptors in HBECs *in vivo*. Using HBECs from 36 women, we show that different progestins have distinct biological activities in the breast epithelium that should be taken into account for more informed choices in hormonal contraception, potentially leading to a reduction of breast cancer incidence.

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