Non-melanoma skin cancer (NMSC) is the most frequent human cancer with continuously rising incidences worldwide. Herein, we investigated the molecular basis for the impaired skin barrier function of organotypic NMSC models. We unraveled disturbed epidermal differentiation by reflectance confocal microscopy and histopathological evaluation. While the presence of claudin-4 and occludin were distinctly reduced, zonula occludens protein-1 was more widespread, and claudin-1 was heterogeneously distributed within the NMSC models compared with normal reconstructed human skin. Moreover, the cancer altered stratum corneum lipid packing and profile with decreased cholesterol content, increased phospholipid amount, and altered ceramide subclasses. These alterations contributed to increased surface pH and to 1.5 to 2.6-fold enhanced caffeine permeability of the NMSC models. Three topical applications of ingenol mebutate gel (0.015%) caused abundant epidermal cell necrosis, decreased Ki-67 indices, and increased lactate dehydrogenase activity. Taken together, our study provides new biological insights into the microenvironment of organotypic NMSC models, improves the understanding of the disease model by revealing causes for impaired skin barrier function in NMSC models at the molecular level, and fosters human cell-based approaches in preclinical drug evaluation. Copyright © 2016 Elsevier B.V. All rights reserved. KEYWORDS: Ceramides; Ingenol mebutate; Non-melanoma skin cancer; Reflectance confocal microscopy; Skin absorption; Tight junctions PMID:27130695