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Consumption of meat containing ractopamine might enhance tumor growth through induction of asparagine synthetase

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There is currently no evidence of the carcinogenic effect of the β-adrenergic agonist ractopamine added in finishing swine and cattle feed for promoting leanness. Nonetheless, it has the capability of stimulating expression of asparagine synthetase (ASNS) through activating transcription factor 5, and many other genes involved in the stress reaction in the skeletal muscle of pigs according to published scientific articles. Because overexpression of ASNS has been detected as a key player in amino acid response and unfolded protein response during the development of not a few malignant diseases, especially those with *KRAS* mutations, and found to be closely related to tumor proliferation, invasion and metastasis, it seems reasonable to hypothesize that intake of ractopamine residue in meat might bring negative effects to cancer patients. ***European Journal of Cancer Prevention*** **31: 82–84 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.**

European Journal of Cancer Prevention 2022, **31**:82–84

Keywords: amino acid response, asparagine synthetase, feed additive, ractopamine

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Received 22 November 2020 **Accepted** 30 November 2020

Introduction

Ractopamine, classified as a β-adrenergic agonist, has been used to increase carcass lean percentage and growth rate by pork and beef producers in the USA in the past two decades. Although its use has obtained approval as a feed additive for swine in the USA, Canada, Mexico and Brazil, ractopamine is prohibited in the European Union, China and many other countries (Vezzoni de Almeida *et al.*, 2012). Despite the potential contribution of ractopamine to environmental protection by increasing efficiency of husbandry, shortening finishing swine growth period, decreasing feed need and thus reducing the amount of water, fertilizer and pesticide for crop cultivation (Woods *et al.*, 2011), people inevitably are still worried about the safety of ractopamine residue in meat especially in those countries where pork is imported from the USA.

In addition to the health risk of cardiovascular functional disorders and cardiovascular diseases which might lead to life expectancy decrease (Zaitseva *et al.*, 2014), another major concern is whether ractopamine will promote malignancy formation. Even with the promise that genotoxicity and carcinogenicity could be excluded with acceptable daily intakes on recommended maximal residue limits of ractopamine in animal food except for a dose-dependent slightly increased incidence of uterine leiomyoma in rat studies (Joint FAO/WHO Expert Committee on Food Additives 2004; Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed 2009), what's the effect of ractopamine on cancer already existing remains a question to be carefully investigated. Herein, a hypothesis is raised that ractopamine might stimulate tumor growth through induction of asparagine synthetase (ASNS) expression.

Metabolic reprogramming in cancer cells

During tumorigenesis and metastasis, being affected by oncogene activation and tumor suppressor inactivation, cancer cells rewire their metabolism to meet the biosynthetic demands of high proliferation rates and achieve a new state of homeostasis, losing expression of some anabolic genes and becoming eagerly dependent on certain specific amino acids which could be deprived by enzymes designed as therapeutic modalities (Maggi and Scotti, 2019; Garcia-Bermudez *et al.*, 2020). Nevertheless, cancer cells could also manage to start initially silent genes in response to the urgency of producing amino acids they need. It has been disclosed that one of these genes activated in cell stress is ASNS.

Asparagine synthetase in tumor biology

ASNS produces asparagine from aspartate in an ATP-dependent process during which glutamine is converted to glutamate at the same time (Lomelino *et al.*, 2017). The basal expression of ASNS is generally low in mammalian organs except in the pancreas and testes as shown in rat experiments and human studies. Nonetheless, transcription of the gene for ASNS could be turned on under stress conditions, such as amino acid response and

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unfolded protein response through activating transcription factor 4 (ATF4) (Balasubramanian *et al*., 2013). For example, clinical investigation has revealed that ASNS expression is much increased in the early stage of development of hepatocellular carcinoma as compared with normal liver tissue (Zhang *et al*., 2013). In tumors with *KRAS* mutations, such as a portion of colon and nonsmall cell lung cancer, activation of ASNS gene seems to be a characteristic presentation (Toda *et al*., 2016; Gwinn *et al*., 2018). Furthermore, asparagine was found to be the key determinant factor governing metastasis potential of breast cancer in a human case. Altering asparagine availability *in vitro*, for example, knockdown of the ASNS gene, strongly influenced the invasive capability of breast cancer cells (Knott *et al*., 2018). Most importantly, extraordinary asparagine dependence was ascertained in mouse soft-tissue sarcoma generated by oncogenic *KRAS* and disruption of *Cdkn2a*. Silencing of the ASNS gene in both mouse and human sarcoma cell lines led to reduction of the percentage of S phase cells (Hettmer *et al*., 2015).

Ractopamine increases asparagine synthetase in skeletal muscles

In a recently published study, skeletal muscle transcriptome analysis in pigs administrated with ractopamine (20 ppm in feed) disclosed remarkably increased gene expressions of ASNS and activating transcription factor 5 (ATF5) but not ATF4 in comparison with pigs injected with growth hormone Reporcin and pigs on ordinary feed (Brown *et al*., 2018). On the other hand, the expression of over 20 ATF4 target genes was induced by ractopamine. The authors concluded that ractopamine treatment resulted in an integrated stress response, peculiarly genes linked to amino acid biosynthesis and protein translation, in the skeletal muscle of pigs.

Hypothesis

Based on its nature as a β-adrenergic agonist, ractopamine surely acts on organs other than skeletal muscle as well. For patients who have malignant diseases including at least hepatocellular carcinoma, breast cancer, soft-tissue sarcoma, colon cancer and nonsmall cell lung cancer, particularly *KRAS*-mutated as mentioned above, consumption of meat containing ractopamine presumably will trigger expression of ASNS and other genes involved in integrated stress response and facilitate tumor growth or metastasis. The induction of ATF5 in the skeletal muscle of pigs provides an alternative pathway of stress response in addition to the well-known ATF4 one. Confirmation of this speculation awaits delicate laboratory investigation, clinical trials and long-term epidemiologic observation but the risk should not be ignored.

Discussion and conclusion

Deprivation of asparagine with bacterial L-asparaginase has been adopted for the treatment of acute lymphoblastic leukemia (ALL) for four decades, taking advantage of the lack of ASNS expression in most ALL. Extension of L-asparaginase use to other malignancies has always been an attractive idea to clinicians as well. However, the main concern would be the correlation of L-asparaginase sensitivity with cellular ASNS content as seen in the studies of ALL, ovarian cancer and NK/T cell lymphoma (Su *et al*., 2008; Lorenzi and Weinstein 2009; Li *et al*., 2014). If ractopamine does stimulate ASNS expression, the strategy of L-asparaginase would be badly hindered in cancer patients who used to consume meat containing ractopamine. Finally, recent research discovered that besides its canonical role of protein synthesis, asparagine may have additional functions in the regulation of cellular growth, prompting reconsideration of the importance of ASNS in cancer therapy (Chiu *et al*., 2020). Accordingly, the presumed negative effects of ractopamine on cancer progression probably will soon become a novel focus of oncology research in the near future.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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