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COMMENTARY

Incretin-based treatment in type 2 diabetes mellitus and risk of cholangiocarcinoma: Is it only adverse drug effect?

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Cholangiocarcinoma (CCA) is a highly aggressive liver epithelial cancer featuring one of the poorest prognosis in human pathology, with < 5% of patients surviving up to 5 years from diagnosis. CCA may arise from both the intra- (iCCA) and the extra-hepatic (eCCA) biliary system, by assuming the merging point of the second order bile ducts as the border. Although much rarer and less studied than the hepatocellular carcinoma, incidence and mortality rate, specifically of iCCA have been progressively increasing in the new millennium. In addition to well-established pre-malignant conditions, primary sclerosing cholangitis and liver cirrhosis above all, other environmental factors have been identified, including toxins (alcohol, tobacco, dioxins, vinyl chloride, asbestos), and drugs (oral contraceptive pills and isoniazid) [1]. A recent large population-based cohort study by ABRAHAMI et al. published in BMJ, expanded the list of potentially dangerous drugs, showing a near doubling of the risk for CCA in type 2 diabetic (T2DM) patients treated with incretin-based drugs, i.e. dipeptidyl peptidase-4 (DPP-4) inhibitors (DDP-4i) and glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonists [2]. By comparing use of DDP-4i and GLP-1R agonists in 154,162 newly treated adults enrolled in a 10-year timeframe, with other second- or third-line antidiabetic drugs, the authors found increased hazards of CCA for either DDP-4i

Abbreviations: GLP-1R, glucagon-like peptide-1 receptor; IL-6, interleukin-6; TNF-α, tumor necrosis factor- α; IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor-1 receptor.

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(hazard ratio, HR = 1.77, confidence interval, CI = 1.04—3.01) or GLP-1R agonists (HR = 1.97, CI = 0.83—4.66). Although the degree of association with CCA was similar, GLP-1R agonists lacked statistical significance because of the wider CI. Of note, in ancillary analysis no association was found with use of insulin, and post-hoc pharmacovigilance analysis confirmed increased odds ratios (OR) for either incretin-based drugs. Since both compounds have been groundbreaking in the treatment of T2DM, this study is indeed of great relevance and open worrisome landscapes in the clinical management of these patients.

GLP-1R was originally identified in pancreatic islet β-cells and then found widely expressed in many peripheral tissues, such as central and peripheral nervous system, lung, kidney, heart, liver and the gastrointestinal tract [3], though in some of them its physiological role is far from clear. In vitro and in vivo data indicate that GLP-1R activation can stimulate cell proliferation and promote resistance to apoptosis, by enhancing cell survival. Proliferative and anti-apoptotic effects of GLP-1R stimulation have been described in several cell types, including pancreatic β-cells [4,5], cardiomyocytes [6], artery endothelial cells [6], thyroid C-cells [7] and remarkably, cholangiocytes [8,9]. GLP-1R-dependent cell tropism can be turned into favorable clinical outcomes derived from improved glycemic control and reduction in cardiovascular events in T2DM patients [10,11]. Unfortunately, the flipside of an 'exaggerated tropism' might be an increased risk of developing malignancies, as addressed by ABRAHAMI's study. In the last few years, the association between incretin drugs and risk of epithelial cancers, i.e. thyroid, breast and pancreas, has been a matter of controversy. C-cell hyperplasia and medullary thyroid cancer were described in rats but not in humans treated with GLP-1R agonists [6,12]. In a large British population-based cohort study, the association of incretin-based drugs with breast cancer resulting from a 'duration-response relation' was explained as the result of a transient increase in the detection of breast cancer in GLP-1 analogue users [13]. Association of incretin use with pancreatic cancer is also debated. A large, international multicenter study showed no association with an increased risk of pancreatic cancer compared with sulfonylureas [14], while another large retrospective cohort study did not find any causal relationships between the two events [15].

Notwithstanding, previous cell biology studies in cholangiocytes provided strong mechanistic support to the conclusions drawn by ABRAHAMI's paper. Interactions between GLP-1 and GLP-1R in cholangiocytes have been extensively analyzed by Marzioni et al. [8,9], starting from the original observation that upon chronic biliary injury, cholangiocytes display neuroendocrine-like features [16,17], and therefore they could respond to neuroendocrine peptides, such as GLP-1. Using in vivo and in vitro approaches, they showed that cholangiocytes constitutionally expressed GLP-1R, and this expression was up-regulated in cholestatic rats following bile duct ligation, an effect counteracted by GLP-1R antagonism with exendin (9-39). Treatment with recombinant GLP-1 stimulated cholangiocyte proliferation through the phospho-inosytol 3 kinase (PI3K)-cAMP/protein kinase A (PKA)-Ca^{2+}-CamKII pathway instead of the classical proliferative mitogen-activated protein kinase (MAPK) signaling acting through extracellular signal-regulated kinases (ERK)1/2/protein kinase C (PKC)α [8]. GLP-1 treatment was also able to prevent cholangiocyte apoptosis, by hampering cytochrome c release and caspase-3 activation [9], a phenotypic trait further proficient to malignant transformation. However, GLP-1 expression by neoplastic bile ducts was not a prognostic biomarker of worse outcome in a cohort of 176 patients with iCCA [18].

Regardless of drug-related effects, it must be underlined that T2DM is emerging by itself as risk factor relevant for several epithelial malignancies, including CCA [19]. In fact, T2DM can increase by 80% the risk of CCA and the increase in CCA incidence and mortality observed over the past 3 decades, parallels the increase in T2DM and metabolic syndrome [19]. Mounting evidence support the notion that insulin resistance is a major determinant of the pro-carcinogenic effects exerted by T2DM [20]. Based on this observation, in the ABRAHAMI's study the real effect of incretin-based drugs might be overshadowed by some potential confounders. Whereas on the one hand some T2DM-associated risk factors for CCA appeared to be more prevalent in patients treated with incretins, on the other hand key risk factors were not considered for stratification in the Cox proportional hazard modeling to evaluate the pro-oncogenic risk of incretin-based drugs. Clearly, ancillary analysis using insulin treatment as a negative control exposure, did not rule out the doubt that incretin-treated patients had indeed a stronger insulin resistance. Indeed, incretin-treated subjects were more commonly obese (body mass index, BMI ≥ 30 in 57.5% of DDP-4i, and 92.7% of GLP-1R agonists vs. 50.4% of patients with second or third-line drugs), and had a much heavier burden of oral anti-diabetic agents (metformin 92.8—97.2% vs. 75.3%, sulfonylureas 66.5—75.7% vs. 41.5%, thiazolidinediones 39.6—54.6% vs. 18%). Both features are consistent with a more severe insulin resistant phenotype. Notably, each 5 kg/m^2 increase in BMI was associated with a 20% increased risk of iCCA, with an overall increase of 62% of the obesity-related risk [19].

That said, T2DM often associates with a variety of chronic liver diseases, in particular with non-alcoholic fatty liver disease (NAFLD), or non-alcoholic steatohepatitis (NASH) if occurring with inflammation, and with liver cirrhosis. The prevalence of hepatitis C virus (HCV) infection is also higher in patients with T2DM than in the general population [21]. In the US, T2DM is nowadays the most common cause of liver disease, and T2DM-related cirrhosis (formerly categorized as cryptogenic cirrhosis) has become the third leading indication for liver transplantation [22]. Notably, development of liver damage in T2DM depends on the level of insulin resistance [22]. Moreover, the core protein of HCV interferes with the insulin receptor signaling, thereby impairing the metabolic effects of insulin [23]. Since all these conditions are well-established risk factors for CCA, information on the prevalence of NAFLD/NASH, cirrhosis and HCV status, that were not available in ABRAHAMI's study, would have been crucial to evaluate the neat impact of incretin-based drugs on the risk of CCA. At least three distinct functional effects potentially relevant for CCA pathogenesis can be related to insulin resistance (Fig. 1) and links T2DM, obesity, and NAFLD/NASH, interwoven conditions clustered in the metabolic syndrome. First, leptin, the adipocyte-released hormone (adipokine) regulating food intake via appetite suppression, which increases with the expanded

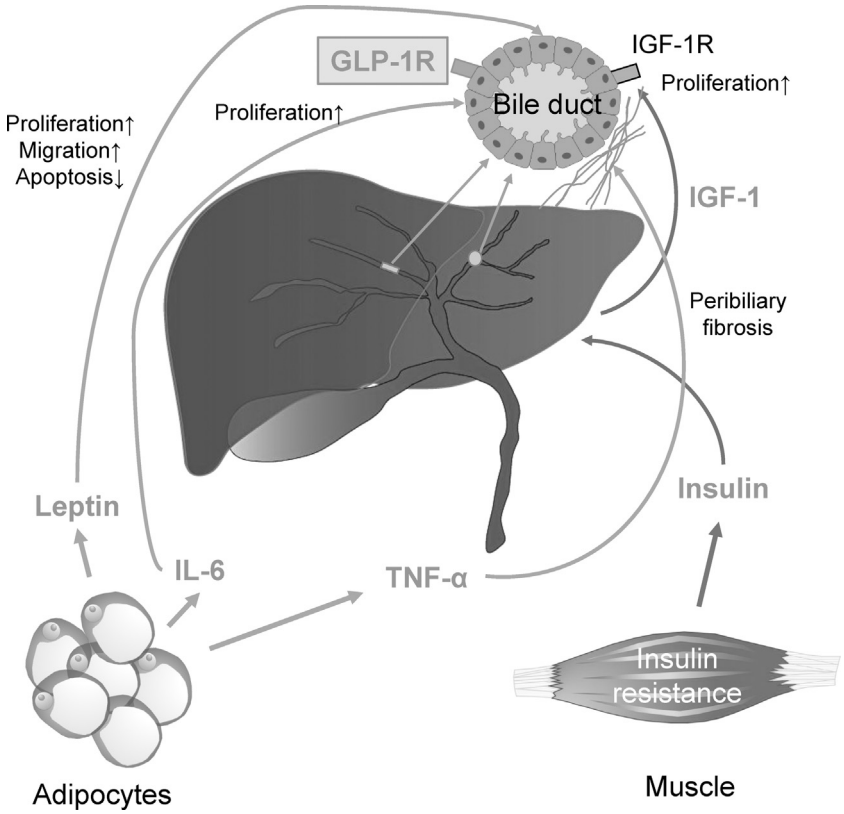

Figure 1 Putative molecular mechanisms cooperating with GLP-1R stimulation to promote biliary malignant transformation in type 2 diabetics with insulin resistance. The cartoon illustrates the different effects on the biliary epithelium exerted by leptin (yellow), low-grade proinflammatory response dependent on IL-6 and TNF-α secretion (purple), and insulin coupled with IGF- 1 liver production (red), potentially synergizing the pro-oncogenic effects of GLP-1R agonists.

adipose tissue mass, is able to stimulate cell proliferation and migration, and to prevent cell apoptosis of iCCA cells in vitro [24]. Second, insulin resistance is associated with the generation of a low-grade systemic inflammatory response with increased levels of pro-inflammatory cytokines, in particular interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [25], which at the biliary level, are potent inducers of proliferation [26] and of a pro-fibrogenic inflammatory milieu [27], respectively. Third, insulin resistance leads to a compensatory systemic hyperinsulinaemia. By activating its specific receptor, insulin regulates two different pathways, one metabolic, governed by the PI3K/AKT signaling, and the other mitogenic, controlled by the MAPK cascade. Noteworthy, in conditions of insulin resistance, response to insulin stimulation of the PI3K/AKT-mediated metabolic signaling is defective, leading in turn to an overactivation of the MAPK-dependent proliferative pathway [28]. Although this unbalance was originally described in the muscle, an insulin-induced pro-proliferative stimulus might be also operating in cholangiocytes, which have been shown to possess insulin receptor [29]. Moreover, in the liver, hyperinsulinaemia is coupled with an increased insulin-like growth factor-1 (IGF-1) production [30]. By activating its cognate receptor (IGF-1R), IGF-1 unfolds gene expression programs promoting cell proliferation and survival [31]. Interestingly, both IGF-1 and IGF-1R are strongly expressed by malignant bile ducts [32]. Clinical relevance of these mechanisms is supported by epidemiological observations indicating that metabolic syndrome significantly increased the risk of iCCA in the general US population (OR = 1.56, CI = 1.32—1.83) [33].

In conclusion, the study by ABRAHAMI et al. outlines the potential detrimental effects of incretin-based drugs, as their potent tropism on GLP-1R expressing cells, including cholangiocytes, may become pro-oncogenic, at least in specific cell-contexts, albeit combinatorial interactions with different factors associated with insulin resistance are conceivably needed for developing CCA.

Disclosure of interest

The authors declare that they have no competing interest.

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