Cytoplasmic-Nuclear Trafficking of G1/S Cell Cycle Molecules: A Novel and Critical Regulatory Mechanism Controlling Adult Human Beta Cell Replication

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Purpose: Adult human ß cells are resistant to attempts at inducing proliferation. Proliferation is controlled by a family of ~30 G1/S molecules (8 E2Fs, 3 pRb members, 4 INK4s, 3 KIP/CIPs, 4 cdks and 7 cyclins, and others), and several (eg., cdks 2, 4, 6, cyclins D1-3, E) can induce adult human ß cell replication. We now have an islet G1/S molecule roadmap, yet since it was derived from immunoblots of whole human islets, it does not document which, if any, of the G1/S molecules are actually present in the human ß cell. Here, our initial goals were to define which G1/S molecules are present in the human ß cell, and to develop an immunohistochemical (IHC) human ß cell G1/S molecule "atlas". We assumed these molecules would reside in the nuclear compartment.

Methods: Using IHC in dispersed human islets, all 30 G1/S molecules, except cyclin D2, were observed in the human ß cell. Surprisingly, however, all were cytoplasmic, and absent from the nucleus, with only three exceptions: pRb, p21 and p57. This was independently evaluated for each G1/S molecule using subcellular fractionation of human islets, which confirmed that all but pRb, p21 and p57 are cytoplasmic, not nuclear, proteins. Most importantly and to rule out any artifact due to islets isolation, we determined the expression and localization of some of these G1/S molecules in intact human pancreas sections. While pRb and p57 were confirmed to be nuclear, cdk6, p18 and p107 were found cytoplasmic in human beta cells.

Summary of Results: We asked whether induction of proliferation might alter the subcellular localization of the 30 G1/S molecules in the ß cell. Adenoviral overexpression of cyclin D3 and cdk6 led to brisk increases (30-50x; from 0.3%, to 10-15%) in adult human ß cell proliferation (BrdU, Ki67). The nuclear presence of cdk6 and cyclin D3 increased dramatically (basal 0%, stimulated ~40%). In addition, p16, p21 and p27 also migrated to the nucleus (basal ~10%-7%-2% respectively, stimulated 20%-35%-15% respectively), whereas other G1/S members remained cytoplasmic. p57 was less frequent in the nucleus (basal ~40%, stimulated 25%). Interestingly, nuclear trafficking of cdk6 occurred early (within 24h) and remained constant for 72-96h, whereas cyclin D3 nuclear entry appeared within 24h, peaked by 48h, and declined precipitously by 72h. Critically, proliferation occurred predominantly in cells that were positive for nuclear cyclin D3 and/or cdk6, and negative for nuclear 16, p21, p27 or p57.

Conclusions: This study provides the first comprehensive human ß cell G1/S "atlas". It shows that all G1/S molecules except for cyclin D2 reside in the human ß cell, and that cell cycle control molecules, widely assumed to be nuclear, are in fact cytoplasmic, but can traffic to the nucleus in association with activation.